EXPERIMENTAL LIVER TRANSPLANTATION

BASIC OBSERVATIONS IN THE PIG

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by

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(vi) *Gastric Ulceration complicating Experimental Liver Transplantation:*
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CHAPTER 1

INTRODUCTION

THE DEVELOPMENT OF LIVER TRANSPLANTATION

Before Liver Transplantation

At the turn of this century Carrell and Guthrie (46) demonstrated that blood vessels could be anastomosed and remain patent. Carrell introduced fine round-body suture needles threaded with oiled linen and defined basic techniques of vascular surgery that subsequently made whole organ transplantation possible. In addition he described differences in outcome between autotransplanted and homotransplanted kidneys, mentioned organ perfusion, and suggested that cooling might increase organ viability.

For the next forty years transplantation remained in the experimental laboratory and it was not until the progressive revelation of immunological principles by Medawar (117, 118) and the demonstration of immunological tolerance by Billingham (26), that interest in transplantation was given impetus. Rapid progress followed in the 1950s. Impairment of immunological response in patients with hypogammaglobulinaemia was reported (47), and in 1955 Hume (84) published an account of successful kidney transplantation in man. Human organ transplantation promised to be an exciting therapeutic possibility.

Early Pioneers

The first attempts at experimental liver transplantation were directed at establishing a satisfactory technique and in crude terms observing the maximal survival of the recipient. Goodrich, Welch and their group (183, 69) set out in 1955 "to find out whether the operation was technically possible with survival of the liver, and, second, to determine the longevity of such transplants and their effect upon the host". Using dogs they performed auxiliary liver transplantation into the pelvis in 49 animals, observing that the procedure was possible, but that only one dog had viable liver tissue for more than 6 days. They reported that "the exudate consisted of numbers of round cells of chronic inflammatory type, and larger mononuclear cells, presumably fibroblasts". They concluded that "it is quite improbable that human livers could be transplanted with survival of the transplants". Cannon (42) briefly reported attempted orthotopic homografts under mild hypothermia, performing " 'successful operations' without survival of the 'patient' ". Three years later Moore and his associates (112, 114) published the results of their recent work. They had faced and solved many technical problems and established an orthotopic technique virtually similar to that used in most centres today. Most significant was their report that the recipient could tolerate short term hepatectomy and that the donor liver could survive short term ischaemia if made hypothermic. Pooling of blood in splanchnic and distal body areas was overcome by shunts from the portal vein and inferior vena cava to the jugular veins. Documenting detailed pathological findings (99) in their 8 animals surviving 4 days, they recorded the canine picture of initial liver function followed by progressive

failure with plasma cell and lymphocyte infiltration and rejection at the end of the first week.

Soon after Moore had embarked on experimental liver transplantation, Starzl (who had recently published papers on hepatectomy technique (138), and with Kaupp on the use of external bypass during hepatectomy (88)) commenced liver transplantation in the dog. He reported in discussion after Moore's paper on the subject to the American Surgical Association in West Virginia in 1960 (137) that he had performed about 80 transplants. With Kaupp he had used a portacaval shunt initially (89), but had found anatomical restoration of the vessels more satisfactory (151). Excluding the first 27 animals of his series, he had 8 survivors of more than 4 days, with a maximal survival of 20½ days.

Improvement of Techniques and Natural History of Rejection Defined in Dogs

In 1961 Starzl recorded in detail (152) the typical picture of liver rejection, observing "complete initial function" and then progressive deterioration of the clinical and biochemical course. He found duodenal ulceration in 6 of his longer surviving 18 animals. With members of his group he amplified his observations by publishing the biochemistry of postoperative hepatic function (90, 91), including a description of the slow decline in albumin and total protein and an increase of β and γ globulin. Histopathological alterations associated with rejection (20) were reported. In addition, the effect of host immunological paralysis by whole body irradiation (146) (which failed to prevent rejection), and graft immunological paralysis by irradiation

(which also failed to prevent rejection and established that the previously recorded host lymphoid changes were not part of a graft versus host reaction) were also studied.

In 1963 Sicular and his associates (134, 135) entered the field and undertook experiments "designed to demonstrate rejection in the absence of hepatic insufficiency and jaundice", by auxiliary transplantation into the pelvis, leaving the host liver undisturbed. They observed rejection of the auxiliary transplant with progressive deterioration of bile flow, infiltration by immunological cells and eventual graft destruction. There was no evidence of graft versus host reaction in the host liver and the host lymphoid organs demonstrated an active immune response. A further publication (122) stated that globulin was found in plasma cells, liver blood vessels, bile ducts and macrophages, and the authors postulated that tissue antigen of a polysaccharide nature entered the circulation from the graft and became localised in the spleen and lymph nodes of the host, producing rapid proliferation of antibody forming cells which then colonised the liver. From these and peripheral cells, antibody was released and directed against the liver. In addition, they found evidence of complement binding in the transplant.

Various technical possibilities were subsequently published: auxiliary transplantation into the splenic bed (78); auxiliary transplantation into the pelvis, with partial shunting of the host portal flow via the splenic vein into the donor portal vein (102); transplantation without external shunts by performing an initial host portacaval shunt,

then leaving the host vena cava intact, followed by restoration of portal continuity (12).

Further impetus was given to liver transplantation research by Eiseman (52) and others, who demonstrated that the liver could be maintained on isolated perfusion for several hours with significant retention of viability.

By 1963 - some 7 years after the first experimental transplant - the natural history of the dog liver allograft had been documented, but published series all recorded high technical mortality with few survivors after 4 days (a stage after which subsequent events could be attributed to the immune response). Out of Moore's series of 35 animals, only 4 survived 5 - 12 days (99); in Starzl's initial series of 80, only 18 survived more than 4 days and over 90 per cent were dead in 5 - 10 days, with maximal survival of 20 days (151). Auxiliary transplantation had yielded results only marginally better (132, 133, 96).

Immunosuppression and Prolongation of Survival

Encouraged by the increasing success in the prolongation of kidney transplant survival by immunosuppression (115, 131, 48), both Starzl and Moore embarked on programmes to prolong liver graft survival. Using immunosuppressive regimes with azothiaprine, azaserine and cortisone, Moore (111) obtained maximal survival of 12 days in 10 animals. Despite lack of clinical survival, immunocyte infiltration was diminished and the important observation was made that graft ischaemia, as demonstrated histologically and angiographically, accompanied the rejection process. Starzl (155, 162) used azothiaprine and prednisone in 25 animals and obtained 10 animals living longer than 7 days,

with a maximal survival of 31 days. He, too, noted a diminution of immunocyte infiltration and was able to support his animals beyond the initial rejection phase, although infection proved a major cause of death in the survivors over a week. In addition, Marchioro and Starzl (95) attempted to evolve a method of donor cadaver perfusion by extracorporeal bypass, in order to maintain donor organ viability after death, while removal was taking place. Half the animals did not survive transplantation and the remainder failed to survive 5 days. Haemorrhage accounted for most deaths and there was "evidence of profound acute liver injury in every case" (162).

Seven Human Transplants Attempted

In 1963 both Starzl (163, 155, 162) and Moore (111) undertook human liver transplantation. At this time over a hundred human renal transplants had been performed with significant prolongation of survival (131). Six terminal patients with biliary atresia or carcinoma received allografts but no patient survived longer than 23 days. Operative death, pulmonary emboli and biliary drainage complications accounted for the mortality. Three patients developed ulceration in the oesophagus, stomach or duodenum. A unique and important body of information was obtained from this experience. The most important was that the human liver could withstand the trauma of transplantation and that immunosuppression was apparently successful - abnormal biochemical parameters tended to improve postoperatively and signs of rejection were never severe (156). Although the human liver was found to be more durable than that of the dog (where congestion - "outflow block" - and progressive hepatic failure follow prolonged anoxic

intervals (95)), it was concluded that a high quality donor organ was essential.(97). In Starzl's cases donor organ viability had been maintained by an extracorporeal bypass technique in the cadaver (95). Prolonged anoxia led to increased coagulation problems, notably marked immediate fibrinolysis followed by hypercoagulability, the latter condition being exaggerated by fibrinogen and E amino-caproic acid (EACA) (182). The technical aspects of the operation had been established and it was found to be unnecessary to use distal venous decompression with bypass in humans. In 1964 Demirleau (166)attempted a transplant in France without operative survival.

In the first 7 human orthotopic liver transplants the maximal survival was 23 days, and the homograft reaction had played no decisive role in the mortality.

In discussion after Starzl's presentation of his cases to the American Surgical Association at Hot Springs, West Virginia, Moore (108) said: "In this rapidly growing field of homotransplantation it is important to recognise things we have not achieved. The long term laboratory survivor is needed and we do not have any! ..... the first successful long term kidney transplantation in man between individuals related more remotely than fraternal twins was preceded by long term survival in the laboratory using a protocol very similar if not identical to that later used in man. .....it would seem wise to slow down and entrench our position by careful laboratory study....."

Return to the Laboratory

During the ensuing 3 years large, meticulously controlled, programmes of liver

transplantation in the dog were undertaken. Authoritative studies were published, notably from the laboratories of Starzl (160) and Moore (168). Operative mortality had been drastically reduced. The natural history of the unmodified dog liver homograft was reconfirmed in biochemical and histological terms and the mean unimmunosuppressed survival time was established at 7.1 days (160). Azothiaprine was found to be hepatotoxic in the dog, producing enzyme elevation within 2 - 7 days after commencing the drug with centrizonal necrosis and hepatocyte abnormalities. Several patterns of rejection were documented, ranging from fulminating rejection in the face of immunosuppression to complete absence of the process. Most significant was the fact that many long term survivors were obtained, 24 animals living more than 50 days, with several survivors after a year in one series (160). Rejection was seen to abate after several weeks in many animals and survival continued after cessation of immunotherapy. Starzl (156) called this condition a state of host-graft nonreactivity, finding that after the initial rejection insult the immunocytes tend to emigrate from the homograft. Groth noted that graft ischaemia was part of hepatic rejection (71, 72, 75).

Yet, with years of formidable experimental experience, together with the use of steroids and azathioprine, the overall mortality rate remained high. The most optimistic expectation in Starzl's laboratory was that between a fourth and a fifth of immunosuppressed operative survivors would live longer than 2 months (160). Moore's laboratory could provide no better results - over half the animals surviving 14 days died of rejection despite their immunosuppression (108). Clearly more long term survivors

were necessary and a more effective, and a less toxic, immunosuppressive agent was required.

In 1963 Woodruff had published his classic report (184) on skin graft prolongation with antilymphocyte serum (A.L.S.), thus focussing new interest on serum that had been a research tool for over half a century (101). Immediate widespread investigation and production of this new immunosuppressive agent followed. By 1966 A.L.S. and its globulin fraction A.L.G. had been shown by two groups (1, 107) to prolong kidney survival in the dog, and the following year Starzl documented extension of survival in this animal after liver transplantation (159). A.L.S. or A.L.G. appeared to have a synergistic effect with azothiaprine (153). Using A.L.S. as the sole immunosuppressant, Birtch and Moore (9) reported an average survival of 54 days in their best group of dogs and noted that rejection played a small role in canine allografts treated this way.

The Pig Liver Anomaly

In the early sixties, during the course of his methodic investigation of the isolated perfused liver, Eiseman found that the pig liver had distinct advantages over that of the dog (51). The biliary and vascular anatomy was similar to that of man and there was no "outflow block", for, unlike the dog, this animal had no hepatic vein sphincters. With Mieny and others he established in the pig observations of function and criteria of viability, as well as techniques for storing the organ for short periods (52, 104, 105, 53).

In 1966 4 laboratories undertook programmes of orthotopic transplantation of the pig

liver and, from their observations, it was to become apparent that liver transplantation in this species differed in many ways from that in the traditional laboratory animal, the dog.

In 1965 Garnier (66) had reported a technique of sham transplantation in this animal, and later his group undertook orthotopic transplantation (43), finding mild rejection in 4 unimmunosuppressed animals living for more than 3 weeks. Two livers at 25 and 51 days showed no stigmata of rejection whatever.

Working in Eiseman's laboratory, Mieny found in 1967 (106) that 4 of 6 pigs surviving more than 5 days died from classical rejection, the mean survival time being 12.4 days. Centrilobular necrosis, bile stasis and round cell infiltration were noted. In this unimmunosuppressed series rejection was a feature, but it was their clinical impression that the unprotected pig tolerated a liver graft better than did the dog.

Simultaneously an extensive liver research programme in Bristol firmly established the pig as a valuable animal in transplant research (124, 173). Peacock and Terblanche recorded the extraordinary phenomenon that, even without immunosuppression, rejection was essentially a subdued process in this animal, both in terms of histology (81) and biochemistry (175). Only 2 animals died of a typical rejection reaction (129) and in some animals there was evidence of reversal of the rejection process. One animal lived for 2 years. Hunt documented in detail the histological picture of rejection in the pig (81). He found that, in the first few days after transplantation, mononuclear

cells were absent, but made their appearance at about the fourth day in the portal and septal areas. Nearly half the cells were pyrinophilic. He also noted the ultra-structural changes in the hepatocyte, chiefly loss of membrane-bound ribosomes and the development of a dilated, rough endoplasmic reticulum. Immunocytes were found in the space of Disse and between hepatocytes. Focal necrosis was minimal and arterial changes absent. A biopsy at 7 months revealed commencing fibrosis. This laboratory maintained that the anomaly of low grade rejection in the pig was a phenomenon of the liver and not the animal, as they were able to demonstrate the animal's immunological competence by normal lymphocyte transfer reaction and skin allograft rejection (87). In addition, they found gastric ulceration to be a frequent complication and observed coagulation changes in this model (11) to be broadly similar to that recorded in dogs and man. Further investigation recorded the rate of temperature rise in the donor liver during its implantation (86). They also observed that the porcine liver could be stored by hypothermic perfusion for 6 hours, without gross demonstrable liver damage (82), and that the use of cadaveric and 5 hour stored livers caused profound metabolic disturbance. In some animals, however, these livers were able to sustain life (123).

In Cambridge, Calne had also turned to the pig as an experimental animal (31, 32, 35,.30). He found that rejection was a slow and remarkably mild process with minimal round cell infiltration in the portal tracts and interlobular septa; he also found that peptic ulceration could be prevented in this animal by vagotomy and gastroduodenostomy. In his initial series 20 animals survived longer than 4 days, none died from rejection

(despite lack of immunosuppression) and 5 animals lived longer than 4 months.

Pursuing the phenomenon of liver graft survival in the immunosuppressed pig, Calne embarked on a series of experiments involving various organ and tissue grafts, with combinations of these transplanted simultaneously (29, 34). He found, like the Bristol workers, that skin was rejected actively. The kidney also appeared to be rejected in the expected fashion. If the pig was sensitized with liver extract or a kidney graft, and a liver transplanted as a second set, active rejection was also seen. However, when the liver was transplanted in combination with another organ, both organs (liver with skin or kidney or heart) underwent a slowing of the expected rejection process. In other words, the liver appeared to confer protection to the simultaneously transplanted tissue. From his data Calne postulated that the transplanted liver produced partial immunological tolerance, by release of "histocompatibility antigens in a tolerogenic form, and that these antigens can induce partial immunological tolerance in immunologically mature animals".

However, not all workers agreed with Calne's thesis of immunological tolerance as an explanation of the anomalous behaviour of the pig liver transplant. Starzl and his group (166) were able to attribute death to rejection in 4 of 7 animals surviving 6 days, and in these the round cell infiltration was dense and both focal and centrizonal necrosis present. While agreeing that rejection in some unimmunosuppressed animals was quantitatively reduced, Starzl denied the fact that this difference was "qualitatively unique", dismissing this concept as a "dangerous notion".

There appeared, therefore, to be a species difference in the nature of liver rejection. The dog and the rat (93) rejected the liver in the "expected" fashion (ie. fatal rejection reaction in 5 - 10 days). Myburgh also found that baboons rejected liver allografts in the expected manner (119). In man only one patient was treated without immunosuppression and this patient underwent fatal classical rejection (185). The pig, however, while rejecting skin and kidney conventionally, reacted in a different fashion when challenged with a liver allograft. Various workers differed in their observations and interpretation of the incidence and immunological significance of this phenomenon.

Human Liver Replacement : An *Interim* Evaluation

By early 1971 over one hundred patients had received liver replacements for various hepatic diseases. Only 10 lived longer than a year. Chronologically there were two recognisable groups: the first was one of 9 patients who received orthotopic transplants prior to July 1967, none of whom survived more than 23 days (163, 155, 162, 156, 166, 111, 108, 182, 95); the second group could be constituted from a surge of interest and activity which took place in 1968, 1969 and 1970, when about 100 patients received liver transplants. Over half the patients were treated by two groups: Calne and his group in England, and Starzl and his group in Denver, Colorado. Their experience is extensively documented (166, 153, 154, 159, 142, 147, 164, 165, 33, 38). The results of other series is scantily documented (166, 9, 41, 63).

At the outset the concept of replacing a diseased liver with a new one held exciting therapeutic possibilities. Potential liver recipients appeared to present a significant

section of the general patient population. Couch (44) computed from the Vital Statistics for the United States that 15,000 people between 5 and 60 died of liver disease in 1963; Terblanche and Riddell (176) calculated that, in ideal circumstances, between 600 and 1,000 patients in England and Wales with cirrhosis, hepatic malignancy or biliary atresia could be helped annually by liver transplantation. In addition, the latter group defined a large group of potential donors which might prove suitable.

The general optimism and enthusiasm felt in 1968, however, had dampened by 1970. The atmosphere of a meeting of all groups concerned with liver transplantation held in Cambridge in 1968 (41) was very different from subsequent meetings in 1970 (186). Earlier profuse and encouraging publications of clinical experience became less evident.

There appeared to be various reasons for this. The calculated donor supply was far in excess of that actually realised; legal and ethical problems emerged (3) and, moreover, unlike the kidney, the liver proved to be incapable of satisfactory storage or transport (166). Perhaps the overshadowing reason was that the first 110 cases of orthotopic liver transplantation had not achieved their earlier expectations.

The rationale for this drastic form of therapy was basically to "prolong life in patients with hepatic disease" (164). Clearly therapeutic success could be said to have been achieved if the patient lived longer (and more comfortably) after transplantation than he would have done without this form of therapy. By 1970 this condition appeared questionable.

The three main disease processes treated were hepatic malignancy, cirrhosis and biliary atresia. Liver replacement for hepatic malignancy appeared logical if the disease were confined to that organ, but it would have to prove more successful than the known survival time for this disease. From selected authoritative series, the mean survival time was a year and a half for patients with carcinoma at the junction of the main hepatic ducts treated by palliative bypass (189); the mean survival time for colon metastases was 13 months (27); the one year survival rate for hepatocellular carcinoma was just under 10 per cent (54). Hepatocellular carcinoma, when amenable to lobectomy, had a one year survival rate of 30 per cent in Lin's hands (94). Liver transplantation would have to provide better results than these. In Starzl's series 6 cases with hepatoma lived through the immediate effects of the operation, but 5 eventually died with evidence of tumour recurrence between 76 and 432 days. One was alive at 9 months without evidence of recurrence (166, 161). In addition, it appeared an immunological possibility that immunosuppression by depression of the total host defence mechanisms could accelerate recurrence. Starzl concluded "in historic perspective, the experience with hepatomas will probably be viewed as significant principally because the efforts at therapy were responsible for demonstrating that the operation of liver replacement could be successfully performed in man" (165). Hepatic malignancy was the indication for transplantation in nearly half the published cases, and no cases fared as well as Starzl's 6 patients.

The remainder of the cases were largely constituted from biliary atresia and cirrhosis.

Prognostication with these diseases is notoriously difficult. Most children with untreated intrahepatic atresia live to the age of 5 years, and survival to the age of 10 has been recorded. With the extrahepatic variety, where conventional surgery has not been possible, the child usually survives for 2 years (145). Of the first 15 cases of atresia treated by Starzl, 2 children died at 13½ and 29 months, and 3 were alive at 9, 12 and 26 months (166, 161).

The total experience with cirrhotics in liver failure was more disappointing. The majority of patients died within days of transplantation. To fulfil candidacy in this category, the patient would have to be demonstrably dying. The prospect of undertaking massive surgery in a patient with prothrombin depression, hypoproteinaemia and the other sequelae of hepatic deterioration, could hardly be expected to be met with a high degree of success. The survival rate after transplantation for cirrhosis was worse than that for hepatic malignancy or atresia (166, 36), although a survival rate of up to a year has been reported (37).

The first 100 orthotopic grafts were performed in various centres throughout the world, with over half the cases undertaken by either Starzl or Calne. The mean survival time of all cases other than those performed by these two groups was a month, with occasional survival for several months (166, 9). These figures contrast strongly with the expertise of Starzl who had 8 of 33 patients survive over a year (161), and with Calne's long term successes (37). The natural history of the human liver transplant may be drawn from the two major series.

During and after transplantation, there was an haemorrhagic tendency (76, 64), which was due to both an increase in fibrinolysis and a fall in clotting factors synthesised by the liver; this tendency was more marked in earlier cases than later ones and was directly related to the quality of the homograft (76). In addition, intravascular coagulation was noted, and Groth was able to attribute most of this effect in the early Denver series to intraoperative efforts to manipulate the clotting mechanism.

The pathological picture of human hepatic rejection is clearly assembled by Porter (126, 128) from his unrivalled experience in the field. During the first 2 - 3 days occasional small lymphocytes are found in the tissue spaces of the liver and large pyroninophilic cells start proliferating in the paracortical zones of draining lymph nodes; at about the third day lymphoid cells start to leave the portal vein tributaries in a random way throughout the graft. The venous endothelium lifts away from the basement membrane and fibrin collects in the subendothelial space. Eventually lymphoid cells accumulate in the portal tracts and central veins, and invade the space of Disse; the infiltration increases, the walls of many sinusoids disintegrate, blood flow decreases and some centrilobular hepatocytes die. Inspissated bile appears in the bile canaliculi and lipid droplets are seen in the hepatocytes around the portal tract. Shortly before death of the recipient, foci of fibrinoid necrosis occur in the walls of the small hepatic artery branches and IgG and complement are seen in the vessel wall. The reduction in blood flow is probably due to damage to the venous and sinusoidal parts of the vasculature. When these changes are retarded by immunosuppression, there is

often collapse of the central part of the lobular reticulin framework, which, in some cases, was followed by hepatic fibrosis.

In analysis, Starzl (166) found that death within the first two months was due either to homograft damage (manifesting as bleeding, hepatic failure or infection), or to technical failure, usually vascular occlusion or biliary complications. Death in the next two months was usually due to hepatic infarction or recurrent malignancy. Rejection was seen to present various clinical profiles: anicteric rejection, rejection crisis, indolent rejection and late cholestatic rejection. Calne's clinical experience was broadly similar (33, 34, 185, 186, 64, 36, 37).

Various new biological phenomena were revealed in the course of this experience. After transplantation a patient with primary biliary cirrhosis showed a fall in titre of serum mitochondrial antibody (186); a patient with hepatoma and tumour parathormone excretion showed return to normal of serum calcium and parathormone levels (185); a patient with Wilson's disease had, at 18 months, cleared his body copper stores without accumulation of copper in the transplant, thus suggesting that Wilson's disease was an inborn error of hepatic metabolism (190). A further patient with "chronic aggressive hepatitis" became Australis antigen negative (having previously been positive) immediately after liver replacement; $2\frac{1}{2}$ months later the test became positive again and there were signs of active hepatitis.

Of the first 110 patients, only 10 lived longer than a year. A unique body of

information had been acquired, but the therapeutic place of liver transplantation remains controversial. Starzl holds that liver transplantation "is a legitimate, albeit imperfect, form of treatment", and that "the prime indication ..... is non neoplastic hepatic disease" (161). Perhaps Moore's observation in 1964, when the first cases were reviewed, is once again pertinent: ".....it would seem wise to slow down and entrench our position by careful laboratory study" (108).

It is against the background of this review that the propositions of the thesis will be placed in the next chapter.

CHAPTER 11

THE THESIS

It is apparent from the preceding introduction that despite considerable laboratory and clinical experience in the field of liver transplantation the overall results of this procedure are far from satisfactory. The exciting challenge of replacing a diseased organ with a healthy one was met with problems of considerable magnitude and diversity. One of the most serious was the fact that the liver is an unpaired organ, for which no adequate artificial substitution therapy exists and failure of a liver graft lead to the death of its recipient. Technical, immunological and metabolic problems clearly have to be solved in the experimental laboratory, as do the vital problems of organ preservation.

The natural history of a liver allograft in the dog model has been extensively investigated and basic data have been established and accepted. The pig liver model, however, has received less detailed investigation. Not only this, but, basic information, far from being established and accepted, has in fact been highly controversial. It was for these reasons that the following study was undertaken in the pig model.

The study was designed to examine the following aspects of unmodified pig liver transplantation:

(i) To define the natural history in clinical, biochemical and histological terms by analysis of repeated blood samples and biopsy specimens taken in the in vivo state, thus establishing correlation between biochemical and histological changes. The controversial role of rejection in mortality could then be assessed.

(ii) To determine abnormalities which were specific to the rejection process, by establishing a complementary study of liver autografts for comparison.

(iii) To determine the most satisfactory form of biliary drainage by testing choledochodochostomy and cholecystduodenostomy in the allograft and autograft models.

(iv) To analyse the complications : biliary stasis, cholangitis, infection and peptic ulceration.

CHAPTER III

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The following experimental groups were established:

1. ORTHOTOPIC LIVER ALLOGRAFTS
   - (a) cholecystduodenostomy (animals 1 - 10)
   - (b) choledochodocostomy (animals 11 - 20)

2. ORTHOTOPIC LIVER AUTOGRAFTS
   - (a) cholecystduodenostomy (animals 21 - 30)
   - (b) choledochodocostomy (animals 31 - 40)

3. CHOLECYSTDUODENOSTOMY CONTROLS (animals 41 - 43)

SELECTION OF PIGS

Three distinct breeds of pig were used:

(i) Landrace breed: (Mr. J.H. Blankenberg, "Klipheuwel Plaas", Klipheuwel, Cape Province)

(ii) Large White breed: (Mr. N. Payne, "Wiltshire Downs", Durbanville, Cape Province)

(iii) Landrace-Large White cross breed: (Mr. G. Sturt, "Eikebome", Somerset West, and Mr. S. Michelson, "Champagne Estate", Somerset West, Cape Province)

The greatest possible genetic disparity between donor and recipient was aimed at in the allograft experiments. The genetic cross is given in Table 2, facing page 45. Another consideration in animal selection was the discovery of Halothane induced hyperpyrexia in the local Landrace pigs (85). These animals were only used as donors in the allograft experiments and the difficult technique of Pentothal anaesthesia employed. All control autograft and cholecystduodenostomy experiments were performed using the Large White breed. Pigs of 20 - 50 kilograms were used. No consideration was given to the sex of the animals in their selection.

ANAESTHESIA

Animals were kept on glucose water in a bare sty for 24 hours preceding operation, to prevent gastric distension at operation and to maintain glycogen stores. No antibiotic bowel preparation or other medication was given. They were brought from the sty to the operating theatre in a mobile cage.

Three methods of anaesthesia were used.

1. Halothane and intermittent positive pressure respiration

The majority of animals were anaesthetised this way. The technique used was that initially described by Dawson (50) and developed by Bowes (174). Induction was with 3 per cent Halothane, nitrous oxide (3 L/minute) and oxygen (5 L/minute), through a nose cone. Pigs were intubated by inserting an endotracheal tube once the vocal cords had been visualised with a long, straight laryngoscope. The tube was rotated through 180° in order to accommodate the natural curvature of the trachea to that of the tube. A large bore stomach tube was also passed. Anaesthesia was maintained with nitrous oxide (3 L/minute) and oxygen (5 L/minute) and the Halothane was reduced as far as possible (0.5 - 1%). Artificial respiration was achieved with either a "Bird" or "Manley" respirator. The mixture of Halothane was temporarily increased if the animal moved during operation, and reduced during the anhepatic period and before the end of the operation.

2. Halothane and spontaneous respiration

For minor procedures (eg. liver biopsy) anaesthesia was maintained with a nose cone.

3. Pentothal induction and intermittent positive pressure respiration

This method was used in the Landrace animals where Halothane induced hyperpyrexia was a possibility. The animals were restrained and Pentothal (1 - 1½ gm.) given into a small ear vein. After intubation anaesthesia was maintained on nitrous oxide, oxygen and occasional intermittent intravenous Pentothal.

OPERATIVE TECHNIQUES

Three operations will be described: Orthotopic liver transplantation, the autograft technique and the method of performing the cholecystduodenostomy controls.

1. ORTHOTOPIC LIVER TRANSPLANTATION

In 1959 Moore et al. (112) outlined the basic technique of orthotopic liver transplantation in which the anhepatic phase in the recipient was linked with venous decompression from the vena cava and the portal vein by bypass lines to the jugular veins. Porcine transplantation techniques have been described by Garnier et al. (66), Peacock et al. (124), Terblanche et al. (174), Calne et al. (35), and by Eiseman's laboratory (106). After examining the literature and having the opportunity to observe the techniques of Calne and Terblanche, the author evolved a technique which will be described in some detail. This technique enabled the surgeon to perform both operations himself with an anaesthetist and a single assistant.

The essence of the procedure was that the liver was skeletalised (reduced to its vascular attachments) in both donor and recipient, both livers were excised and the donor liver anastomosed into the recipient hepatic fossa. Important considerations were reduction to a minimum of liver ischaemic and portal bypass times.

In the technique to be described, there were six stages and each stage took approximately half an hour, thus making the total operating time about 3 hours, and the average total ischaemic time to the liver just over an hour.

STAGE	DONOR	RECIPIENT
1.	Skeletalise Liver	
2.		Skeletalise Liver/Bypass
3.	Bleed out Pig Prepare Thoracic Aorta	
4.	Removal and Perfusion	Removal of Recipient Liver Portal & Suprahepatic Anastomoses Portal Flow
5.		Infrahepatic & Aortic Anastomoses
6.		Bile Duct Anastomosis & Closure

STAGE 1 (Donor operation)

A midline incision from xiphisternum to 4" above the pubis (deflecting laterally to avoid the urethra in males) was made down to the peritoneum through the linea alba. The peritoneum was opened. The assistant retracted the bowel inferiorly and to the left, while the operator elevated the right lobe of the liver in his left hand, exposing the peritoneal attachment of the liver and IVC to the posterior abdominal wall. With the right hand he coagulated any small vessels in the peritoneal fold and then incised it from the adrenal below to the suprahepatic IVC above. The liver was replaced and attention was turned to the infrahepatic cava. If this was of adequate length, nothing further was done; if, however, liver tissue extended inferiorly on the cava, dissection was continued inferiorly and medially by incising the peritoneum reflected from the cava to the pancreas. The adrenal was left undisturbed.

Isolation of the portal tract

With the stomach retracted downwards and the liver upwards, the gastrohepatic omentum and portal tract were exposed. The anterior layer of this omentum was incised from the foramen of Winslow to the oesophagus in plane C over the upper border of the stomach (Fig. 1).

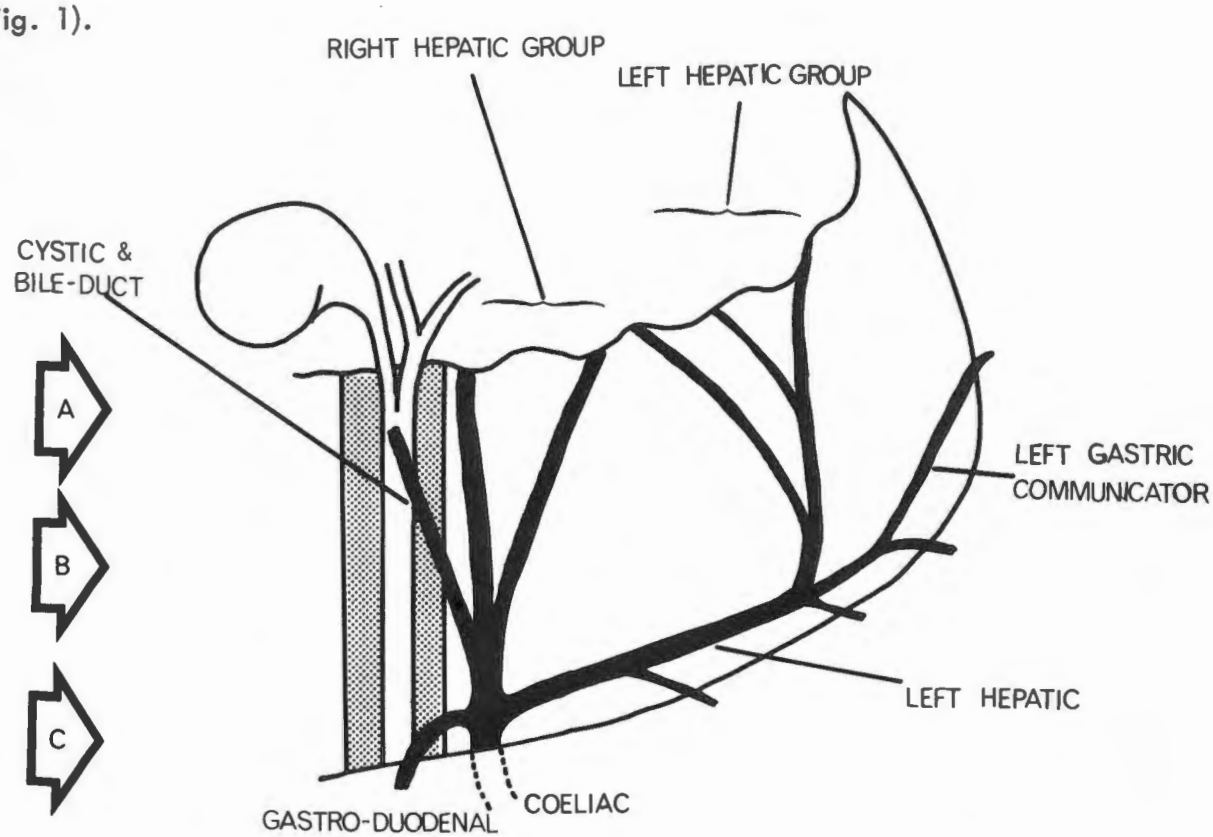

FIGURE 1: The main arteries in the gastrohepatic omentum, and the directions of dissection.
A. Recipient (To preserve gastric blood supply).
B. Autograft.
C. Donor (To preserve entire hepatic blood supply).

The bile duct was severed without ligation above the duodenum, and in succession the gastroduodenal artery and two or three veins entering the portal vein from the duodenum and pancreas were undermined and ligated. The portal vein was cleaned of attached lymph nodes and their small feeding vessels coagulated.

Isolation of coeliac artery and aorta

A rubber sling was introduced around the coeliac artery. The assistant retracted the stomach and duodenum downwards with his left hand and supported the liver with his right; the operator gently pulled on the arterial sling in an upwards direction, thus exposing the coeliac axis as a ridge behind the peritoneum of the lesser sac. The peritoneum over the artery from the duodenum to the right crus of the diaphragm was then incised and the origin of the coeliac axis identified at the aorta. The artery was surrounded by a further sling at its origin. Gentle traction on both the upper and lower slings simultaneously put all the remaining tributaries of the coeliac artery on stretch and readily identifiable for ligation. The origins of these tributaries were individually clamped (splenic, unnamed gastric, pancreatic and phrenic) and ligated, but their distal ends were usually taken in a single, large clamp with their surrounding tissue and ligated.

Incision of the hepatic ligaments

The operator retracted the liver to the left and incised the left triangular ligament to the suprahepatic vena cava; with the liver retracted downwards the small, tenuous falciform ligament was also incised down to this vessel. The contents of the abdominal cavity were then covered with a warm, moist swab.

This part of the procedure usually took about half an hour and attention was then turned to the recipient pig which had been anaesthetised during this part of the donor operation.

STAGE 11 (Recipient operation)

In addition to the incision described in the donor, the femoral artery was exposed and cannulated for blood pressure monitoring, and a vertical 4" incision was made in the neck to expose the jugular vein which was freed from its bed for a distance of 5 cms. The subclavian vein, which enters the jugular vein at the root of the neck, was cannulated with a polythene cannula which had been primed from an attached sterile drip set. Intravenous fluid administration was now commenced.

Insertion of by-pass lines

The assistant held the spleen downwards and the operator divided the two short gastric vessels between clamps. The omental attachments of the spleen to the stomach, left crus and posterior abdominal wall were cut, leaving the spleen attached at its hilum. The pig was heparinised (1 mg./kilo) and the bypass lines prepared by attaching two Nilaton plastic thoracic catheters to a T-piece, $\frac{1}{4}$" I.D., the third arm of which was connected to a sterile drip set for priming with heparinised saline. No attempt was made to dissect the peritoneum off the splenic vein, nor to ligate any branches. All the structures in the hilum were clamped with a vascular clamp and a noose of thread was placed below the clamp. With the spleen devascularised, one end of the bypass catheter was guided into the vein through a venotomy, the clamp released and the catheter advanced about 2" along the vein on the posterior abdominal wall to prevent obstruction and kinking. The noose of thread was tightened around the hilum, thus holding the catheter in place and occluding the splenic artery. A further loop of

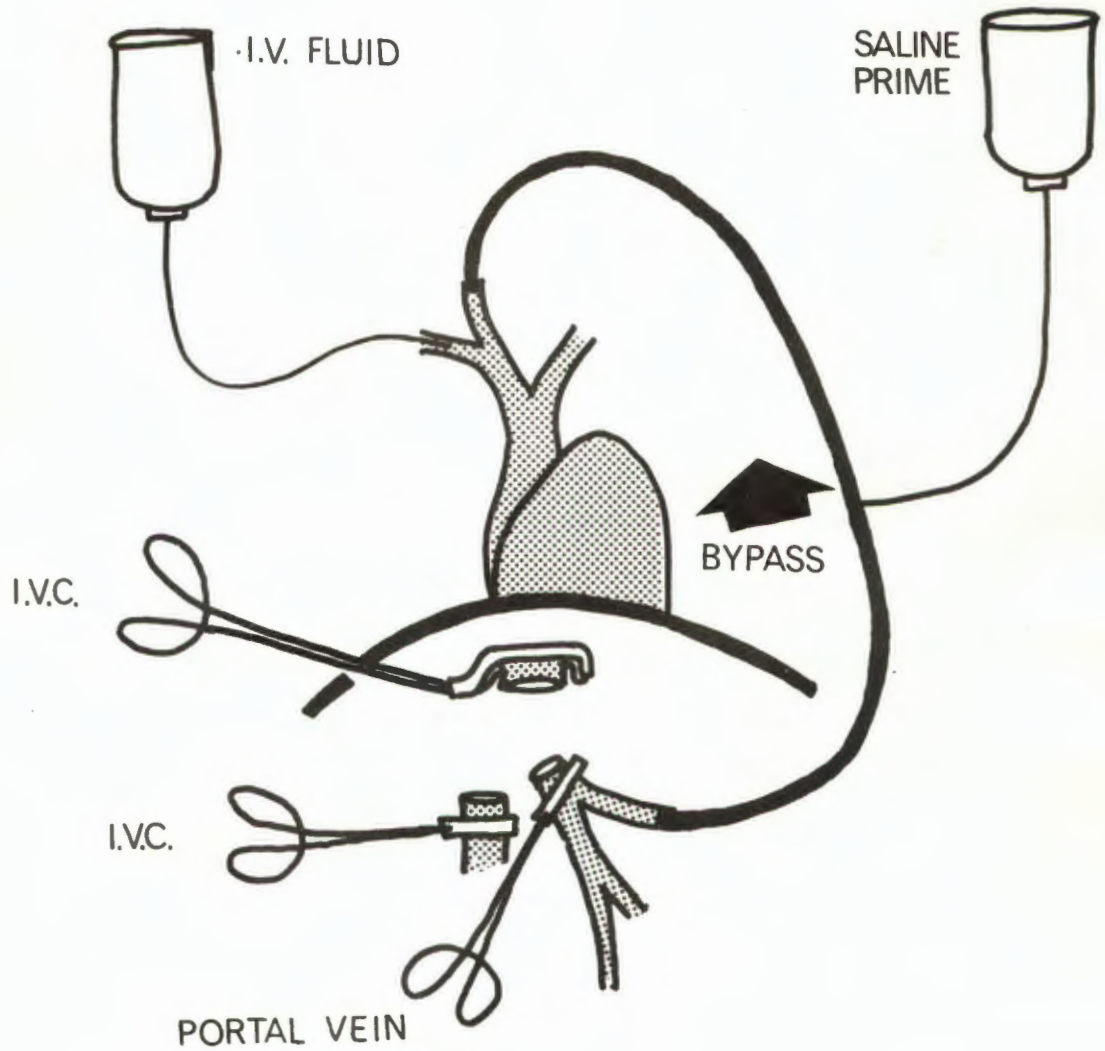

FIGURE 2: Diagram of bypass lines.

thread was blindly introduced behind the vascular bundle at the level of the venotomy and tied around the catheter to prevent movement of the catheter and venous oozing from the devascularised spleen. The primed cervical end of the bypass was inserted through a venotomy in the jugular vein (Fig. 2).

Isolation of the portal tract

Unlike the donor operation, where the overriding consideration was to preserve every arterial branch to the liver, the object in the recipient was to preserve the blood supply to the stomach. The anterior layer of the gastrohepatic omentum was incised next to the liver as indicated (Plane A, Fig. 1, page 27). The bile duct was severed at this level without ligation, and the portal vein was freed from adjacent tissue by blunt dissection and cleaned for 2 inches. A loose sling was put around the portal vein. In order to prevent hepatic ischaemia at this stage, no attempt was made to ligate the artery. The bowel was covered and attention returned to the donor.

STAGE 111 (Donor operation continued)

The donor aorta above its bifurcation was rapidly cannulated with the plastic tube of a Fenwal Transfer Pack TA 10 and exsanguination commenced. The operator stood on the left of the animal and, with the assistant retracting bowel and liver over to the right, incised the peritoneum over the left crus of the diaphragm, the left diaphragm itself and the pleura along the length of the thoracic aorta, thus exposing the aorta from the coeliac origin to below the arch. No attempt was made at haemostasis. Single haemostatic clamps were placed on the aortic side of the intercostal vessels and the first lumbar artery, and the vessels were cut on the thoracic side of the clamp. Oozing from the open end of the vessels was ignored. At this stage exsanguination was practically complete, and perfusion of the liver was commenced by clamping the inferior end of the portal vein and by inserting a primed catheter through a transfer venotomy towards the hilum of the liver. (The cannula was attached to a perfusion system to be described shortly).

STAGE IV

The liver was quickly removed by severing in succession - the aorta below the arch and below the coeliac axis, the suprahepatic vena cava at its junction with the diaphragm, and the portal vein below the catheter and the infrahepatic vena cava above the adrenal. No arterial clamps were used in this procedure and the only precautions against air embolism were taken during the insertion of the portal vein catheter. The liver was gently removed to a sterile bowl where perfusion was continued, covering the organ. During this time the intercostal vessels were ligated and the aorta was cannulated and filled with saline under pressure. Any leaking intercostal vessels were ligated. The suprahepatic vena cava was trimmed to leave about half a centimeter of vessel circumferentially. The liver was left submerged in its perfusate. The operator now returned to the recipient, unclamped the bypass and doubly occluded with vascular clamps the following vessels:

(i) the portal vein at the site of the sling

(ii) the infrahepatic vena cava at its entrance into liver tissue

(iii) the hepatic arterial complex as it entered the liver
(The vessels on the stomach side were individually clamped and ligated; large clamps without ligation were used on the liver side).

(iv) the suprahepatic vena cava
(This was occluded with a single curved Potts Satinsky vascular clamp, including a rim of diaphragm in the clamp. This last manoeuvre was achieved by downward retraction of the liver by the operator's left hand, with the suprahepatic vena cava lying between thumb and forefinger. The clamp was carefully positioned with the right hand.

The vessels were severed, the liver discarded and the donor liver placed in the hepatic fossa.

Anastomoses

(a) *Suprahepatic vena cava* A single, double armed 0000 silk suture was used, anchoring the vessels together at the corner furthest from the operator, with the knot outside the lumen, putting one end of the suture aside and re-entering the lumen on the diaphragmatic side. The posterior layer of the anastomosis was performed from inside the vessel with a simple over and over suture, the stitches being 2 - 3 mm. apart. No clamp was used on the liver side of the lumen. By eversion of both anterior and posterior suture lines the liver was approximated tightly against the diaphragm. Once the posterior suture had traversed and fashioned the corner nearest the operator, it was fixed and the ventral layer rapidly completed with the remaining arm of the suture, using a simple over and over technique and tying the two arms together at completion.

(b) Portal venous anastomosis Both ends of the vessel were trimmed (the liver side to discard the segment where the cannula was tied and the recipient side to avoid kinking due to excess length). A 00000 double armed silk suture anchored the anastomosis at the further corner and a loose stay suture was inserted at the nearer corner. The posterior layer was completed by an over and over everting suture, and the anterior layer with the remaining arm. Just before completion of the anastomosis the portal clamp was removed to allow escape of air. The vascular clamp on the liver side of the portal vein was then removed and portal blood allowed to fill the liver. About 50 ml. of blood was allowed to flow out of the infrahepatic vena cava which was then clamped. The suprahepatic vena caval clamp was then removed, thus reconstituting the portal circulation through the liver into the general circulation. The bypass line was then clamped.

STAGE V (Completion of anastomoses)

(c) Infrahepatic vena caval anastomosis This was performed in the same manner as the portal anastomosis.

(d) Aortic segment anastomosis The assistant retracted the bowel to the left and the abdominal aorta was exposed by incising the peritoneum, medial to the vena cava below the right renal vein. Blunt dissection exposed the aorta (an attempt was made to avoid cutting the lymphatic channels adherent to it), and a curved instrument insinuated around the vessel, from the caval to the left side, to avoid caval tributaries. A sling was placed around the aorta and, by pulling ventrally, a Satinsky clamp was applied to isolate a 2 inch segment of aorta. It was unnecessary to ligate lumbar vessels as these were occluded by the clamp. A longitudinal arteriotomy was made and a 2 mm. rind of vessel wall trimmed on each side with curved scissors. The aortic segment was positioned and trimmed to the correct length. The anastomosis was performed with a double armed 0000 silk suture. The assistant held the aortic segment and bowel to the left with one hand and followed with the other. An over and over suture from above downwards completed the right side of the anastomosis and the suture was fixed inferiorly. The assistant then allowed the partially anastomosed aortic segment to fall into the right paracolic gutter and the left hand side of the anastomosis was rapidly completed with the other arm. The donor coeliac artery was clamped with a bulldog clamp and the aortic clamps removed, thus filling the aortic segment. Contained air was allowed to escape through the open end of the segment, which was then ligated adjacent to

the origin of the coeliac artery. The bulldog clamp was then removed allowing arterial perfusion of the liver.

(e) Choledochodochostomy After trimming the donor end of the bile duct, the anastomosis was performed with a single layer of interrupted 4.0 silk suture.

(f) Cholecystduodenostomy Continuous chromic catgut 000 sutures were employed. A loop of the first and second part of the duodenum was occluded in a soft bowel clamp and the continuous posterior serosal layer of a double layer anastomosis inserted. An adjacent 4 cm. duodenotomy and cholecystotomy were performed and an all coats suture inserted continuously, followed by completion of the serosal layer.

(g) Closure All anastomoses and raw surfaces were checked for complete haemostasis, the peritoneal cavity irrigated with saline and closure commenced. The bypass lines were removed and splenectomy performed. The intravenous catheter in the subclavian vein was retained and brought out through a dorsal neck wound. A single layer closure was performed using continuous 000 nylon through the peritoneum and linea alba: the skin was closed at all sites with a continuous nylon mattress suture.

OPERATIVE TECHNIQUES

2. AUTOGRAFT TECHNIQUE

The preparation of the animal and the operative technique used was exactly the same as in Stage 11 of the allograft operation, except that the ventral layer of gastrohepatic peritoneum was incised midway between stomach and duodenum (Plane B, Fig. 1, page 27). The arterial inflow vessels were carefully and meticulously dissected free of surrounding lymphatics and nerves. The dorsal layer of the gastrohepatic omentum was incised serially between the vessels from the portal vein to the diaphragm and the portal vein was cleaned of peritoneum, nerves, lymphatics and lymphnodes for a distance of about 2 inches. The bile duct was severed midway between the liver and the duodenum. In this way the liver hung, connected solely by its vascular attachments.

Isolation of the liver and its perfusion

Each hepatic arterial branch was clamped with a small bulldog clamp, the portal vein and infrahepatic cava with larger vascular clamps, and the suprahepatic cava with a Potts Satinsky clamp in the manner of the allografts. The bypass was opened. Transverse venotomies were made of the liver side of the clamps of the infrahepatic vena cava and portal vein. The former accommodated a cannula for egress of perfusate and the latter for the infusion cannula. The cannulae were tied in place with loose ties of umbilical tape. Perfusion was commenced through the portal vein, passing through the liver and out through the caval cannula (Fig. 3). Dry swabs were placed above and below the liver to insulate it from the rest of the animal. Once perfusion was complete the portal infusion line was removed and the venotomy closed with 000 silk.

In the experimental design of these autografts, 60 minutes elapsed between hepatic isolation and recirculation. The clamps on the arteries and the portal vein were then removed and the liver revascularised. Approximately 100 ml. of perfusate and blood were allowed to flow out of the caval cannula, which was then clamped and the supra-hepatic clamp removed, allowing circulation into the animal. The caval cannula was then removed and the venotomy closed. A choledochodochostomy or cholecystenterostomy was performed and the animal closed in the manner of the recipient allograft.

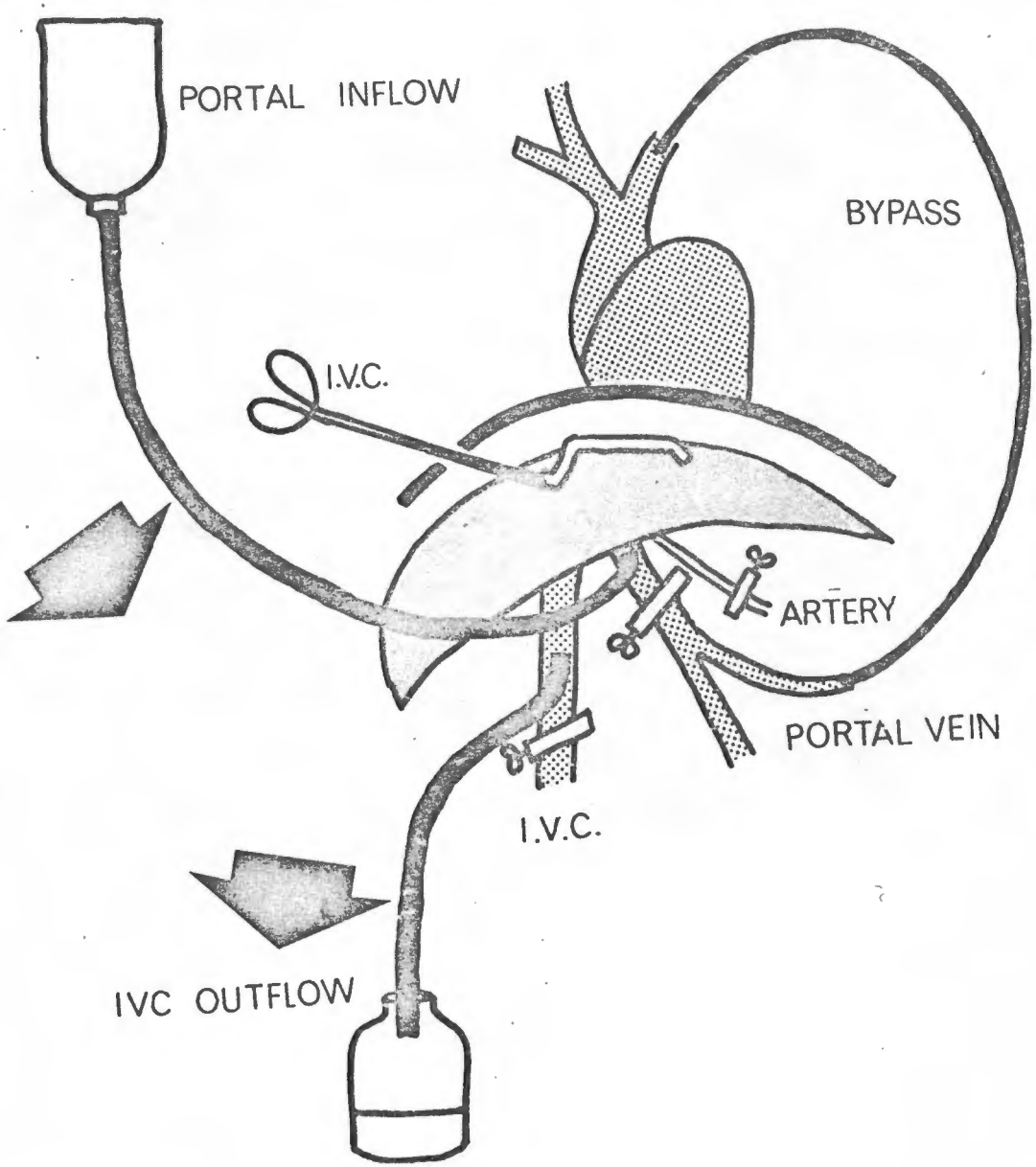

FIGURE 3: Diagram of autograft technique.

TABLE 1 DETAILS OF TIMING AND FLUIDS

Group	Pig No.	TIMES (minutes) Bypass Time	Portal Ischaemic Time	Total Ischaemic Time	INTEROPERATIVE FLUIDS (ml) Blood	Ringer Lactate	$NaHCO_3$
1a	1	80	90	110	3500 (Bank)	1500	210
	2	110	75	105	2500 (Bank)	3500	300
	3	65	65	115	2000 (Bank)	2000	100
	4	39	55	80	900	30	370
	5	25	40	70	320	95	220
	6	35	50	70	400	200	100
	7	28	37	65	300	600	200
	8	30	75	105	300	370	200
	9	35	60	110	300	950	200
	10	31	51	68	450	900	200
1b	11	30	50	80	350	500	150
	12	40	50	65	60	50	300
	13	20	30	60	500	550	300
	14	31	41	70	550	550	200
	15	28	40	70	700	250	250
	16	35	60	75	400	1000	100
	17	25	20	50	500	700	200
	18	25	20	50	300	500	200
	19	38	30	55	500	500	200
	20	35	30	50	200	400	200
11a	21-30	60	60	60	800*	400*	200*
11b	31-40	60	60	60	350*	600*	200*

*Average values
Bank blood was collected from multiple unknown abattoir donors; the remaining animals received donor blood.

OPERATIVE TECHNIQUES

3. CHOLECYSTDUODENOSTOMY TECHNIQUE

The approach was through a midline or a right subcostal incision and, after the common bile duct had been cut and ligated, a cholecystduodenostomy was performed in the manner described in the allograft technique.

LIVER PRESERVATION (Table 1)

Portal vein perfusion was commenced with the heart still beating and the liver receiving arterial blood.

1. Perfusate

In the first 3 allograft experiments and in the autograft experiments 6 litres of Ringer Lactate* were perfused. In the remaining allograft experiments the perfusate was 1800 ml. of TisUsol** with 200 ml. of Macrodex*** added.

2. Ischaemic Time

The total ischaemic time (portal vein and hepatic artery) in the autograft series was 60 minutes. In the allograft series the mean portal vein ischaemic time (portal vein occlusion in the donor to reconstitution of portal flow in the recipient) was 45.5 minutes. The total liver ischaemic time (from initial portal occlusion to total recipient reconstitution) was 68 minutes. The mean bypass time was 31 minutes.

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* *Ringer Lactate: mEq/L - Na 130, K5.4, Mg 2, Ca 1.8, Cl 112, lactate 27.*

** *TisUsol (Baxter Laboratories): mEq/L - Na 137, K 5.8, Cl 142.3, Mg 1.6, So4 1.6, Po4 1.1, with dextrose.*

*** *Macrodex (Pharmacia, Sweden): Dextran mol. weight 60,000.*

3. Temperature

The average perfusate temperature was 8°C. and the average core temperature of the perfused liver was 10°C. An example of heat exchange is given in Fig. 4.

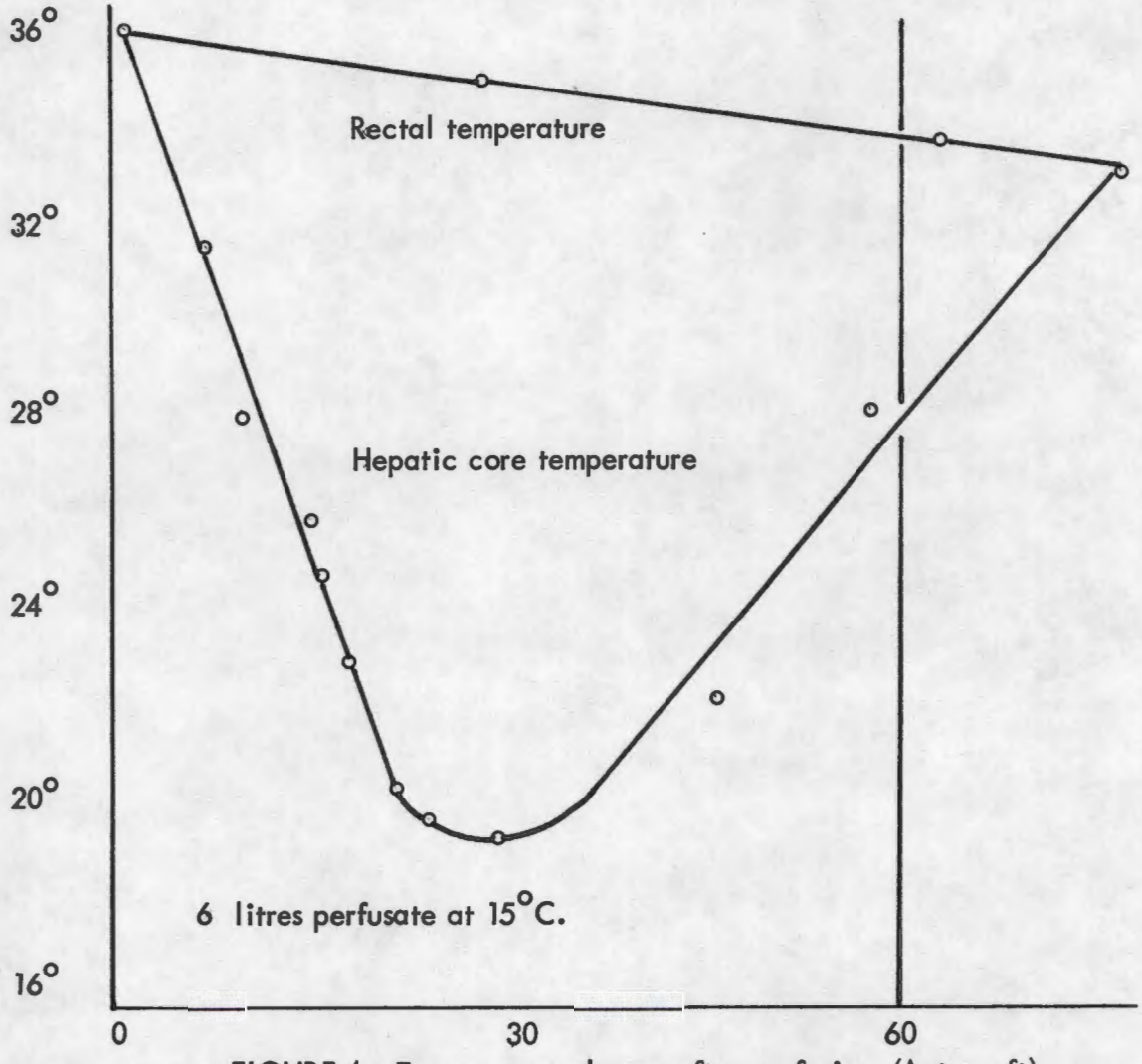


FIGURE 4: Temperature change after perfusion (Autograft)

Comment: Hepatic warm ischaemia was extensively reported by Immelman et al. (86), who recommended liver insulation. In the present autograft series swab insulation was used and no liver liver exceeded 30°C. in an hour. The allografted livers were in place for 30 minutes before portal revascularisation (Table 1, page 38), not exceeding 25°C.

INTEROPERATIVE MONITORING

Arterial blood pressure was monitored with a direct arterial line. In the recipient this remained constant before and after bypass, but dropped during the bypass period, which usually lasted half an hour.

Venous pressure was monitored in early experiments but was found to give disturbingly low readings on all occasions, including an occasion when a pig was deliberately over-transfused with over a litre of fluid. It was therefore abandoned as misleading in the pig.

E.C.G. was employed when this facility was available. Tachycardia with a reduction in voltage was a feature of the bypass interval. Bypass failure or prolonged bypass times were marked by sinus bradycardia, various conduction blocks and, finally, cardiac arrest. Similar features were seen during long operations on cold days when the recipient temperature fell below 30°C. The E.C.G. changes were found to be irremediable by various drugs (calcium gluconate, digoxin, isoprenalin).

Acid base determinations were frequently performed and metabolic acidosis was found after the bypass period (presumably from stagnant anoxia) and after long operations.

Blood glucose estimations were initially performed but found to be of little value as the animal was maintained on a glucose drip.

All these parameters were of use in the initial experiments when operation times and bypass times were prolonged and blood loss significant. Once the techniques became established and standardised, however, they proved of little interest or value. The only remediable situation was haemorrhage, which was immediately obvious to the

operator without its late effects on blood pressure, E.C.G. and acid base balance. All other situations were regarded as irremediable, particularly inadequacy of the portal bypass, in which situation the only remedy was restoration of portal flow by completion of portal anastomoses.

FLUID AND DRUG ADMINISTRATION (Table 1, facing page 38)

Ringer lactate in 10 per cent Invert sugar was administered in approximately equal quantities before and after portal bypass to a total of about 500 ml.

Blood An empirical administration of a pint of donor blood proved sufficient. (The discarded recipient liver was presumed to contain 200 cc. of blood; recipient blood loss was calculated to be about 300 cc.) This was transfused during the bypass period to counteract the diminished venous return during this time. When haemorrhage occurred further blood was administered. The autografts received a similar quantity.

Sodium Bicarbonate Initially alkali administration was calculated from the various acid base determinations, but in the autografts and the latter half of the allografts a standard infusion of 100 mEq. during the bypass period proved adequate to compensate for any metabolic acidosis.

Heparin was given before the introduction of the bypass (2 mg./Kilo) and neutralised with protamine (2 mg./Kilo) after revascularisation in the last 35 allografts and in all the autografts.

Intramuscular chloromycetin was administered before the animal left the table.

AFTERCARE AND ASSESSMENT

First 24 postoperative hours The pigs were removed from the operating table to a cage large enough to allow them to stand, but small enough to allow easy access to the animal and to discourage them from walking and becoming entangled in their drip lines. In the early animals the venous pressure and blood glucose levels were monitored, but in the latter half of the series 1 - 2 litres of dextrose solution as Ringer lactate in 10 per cent Invert sugar was administered empirically.

Return to the sty After 24 hours the pig was returned to the sty and given commercial pig food and water. Five hundred mg. Chloromycetin succinate was given intramuscularly daily for 5 days to all animals. No immunosuppression was employed.

Assessments Blood samples were drawn as required from the intravenous catheter during the first 5 days. This was then removed. At weekly intervals all animals were anaesthetised with a nose cone, weighed and blood drawn from the jugular vein by percutaneous puncture. A liver biopsy was taken percutaneously with a Menghini needle. In certain experiments cultures were taken from the normal liver and duodenum. Cultures were taken from the liver at autopsy and any evident infections were cultured. A full autopsy was performed as soon after death as possible and tissues preserved in formal saline.

Processing of blood and pathological material All blood samples were taken into heparinised tubes for biochemistry, or sequestrin tubes for haematological study. The former were spun, the plasma decanted and deep frozen to await processing. The Department of Chemical Pathology performed the following estimations - alkaline phosphatase, bilirubin, urea, cholesterol and electrolytes. The Liver Laboratory

performed the following estimations - total protein, albumin, S.G.O.T. and L.D.H. The Department of Haematology performed the following investigations - white cell count, platelet count and packed cell volume. The author performed occasional haematological processes, but no biochemical processes. All pathological specimens were preserved in formal saline and processed by the Department of Pathology.

DATA COLLECTION, ANALYSIS AND STATISTICS

Approximately 10 series of observations were made on each animal.

Clinical:	Appearance, weight.
Haematological:	White cell count, PCV, Hb, platelet count.
Biochemical:	Bilirubin, alkaline phosphatase, cholesterol, S.G.O.T., total protein, albumin. (Occasional L.D.H., electrolytes, urea).
Pathological:	Liver biopsies, autopsy, bacteriological culture.

All data was arranged into periods (eg. day or week) for respective groups, thus total information for groups made patterns of behaviour apparent. Mean values were obtained for all groups for all observations. The standard error of the mean and the significance were calculated.

CHAPTER IV

RESULTS AND CORRELATIONS

1. SURVIVAL AND CAUSES OF DEATH
2. CLINICAL OBSERVATIONS
3. BIOCHEMISTRY
4. HAEMATOLOGY
5. HISTOLOGY
6. BACTERIOLOGY
7. CORRELATIONS -
   - (i) Histology and biochemistry
   - (ii) Cholestasis, cholangitis, biliary drainage and rejection
   - (iii) Gastric ulcers, cholestasis and transplantation

Table 2 –SURVIVAL TIMES, CAUSES OF DEATH, AND AUTOPSY FINDINGS IN THE EXPERIMENTAL ALLOGRAFT SERIES (GROUP 1)

BILIARY DRAINAGE	PIG NO.	GENETIC CROSS	SURVIVAL (days)	PRIMARY CAUSE OF DEATH	AUTOPSY FINDINGS			
					Gastric* Ulcer	Rejection	Cholestasis	Cholangitis
a. Cholecystoduodenostomy (gall-bladder to duodenum)	1	Litter mate	51	Infection	+	+	+	–
	2	Cross breed	5	Rejection	(+)	+	–	+
	3	Litter mate	46	Peptic ulcer	(+)	+	–	+
	4	Cross breed	6	Rejection	–	+	+	+
	5	Same breed	21	Pneumonia	+	+	+	+
	6	Same breed	7	Peptic ulcer	+	+	+	+
	7	Same breed	13	Peptic ulcer	+	+	+	+
	8	Litter mate	28	Peptic ulcer	+	–	+	+
	9	Litter mate	8	Peptic ulcer	+	–	+	+
	10	Cross breed	7	Peptic ulcer	+	+	+	+
b. Choledochocholedochostomy (bile-duct to bile-duct)	11	Cross breed	13	Infection	(+)	–	–	–
	12	Cross breed	33	Peptic ulcer	+	–	–	–
	13	Cross breed	18	Peptic ulcer	+	–	–	–
	14	Cross breed	15	Peptic ulcer	+	+	–	–
	15	Cross breed	13	Peptic ulcer	+	+	–	–
	16	Cross breed	7	Rejection	–	+	–	–
	17	Cross breed	23	Unknown	–	+	–	–
	18	Cross breed	24	Peptic ulcer	+	+	–	–
	19	Cross breed	38	Hernia	–	+	–	–
	20	Cross breed	15	Peptic ulcer	+	+	+	+

* Brackets indicate microscopic ulceration only.

Table 3 –SURVIVAL TIMES, CAUSES OF DEATH, AND AUTOPSY FINDINGS IN THE CONTROL AUTOGRAFT SERIES (GROUP 2)

BILIARY DRAINAGE	PIG NO.	SURVIVAL (days)	PRIMARY CAUSE OF DEATH	AUTOPSY FINDINGS		
				Gastric* Ulcer	Cholestasis	Cholangitis
a. Cholecystoduodenostomy (gall-bladder to duodenum)	21	17	Infection	+	+	+
	22	75	Sacrifice	–	–	–
	23	6	Pulmonary embolism	–	–	–
	24	7	Peptic ulcer	+	+	+
	25	6	Peptic ulcer	+	+	+
	26	12	Peptic ulcer	+	+	+
	27	35	Pneumonia	+	+	+
	28	9	Peptic ulcer	+	–	+
	29	27	Infection	+	+	+
	30	28	Infection	+	+	–
b. Choledochocholedochostomy (bile-duct to bile-duct)	31	28	Pneumonia	–	–	–
	32	24	Pneumonia	–	–	–
	33	17	Pneumonia	–	–	–
	34	16	Unknown	(+)	+	–
	35	21	Pneumonia	–	–	–
	36	18	Unknown	–	–	–
	37	18	Pneumonia	–	–	–
	38	17	Pneumonia	–	–	–
	39	22	Pneumonia	(+)	+	–
	40	76	Sacrifice	–	–	–

* Brackets indicate microscopic ulceration only.

FIGURES 2 and 3: By permission of the Editor, British Journal of Surgery.

1. SURVIVAL AND CAUSES OF DEATH

The survival times and primary causes of death of the allografts and autografts are presented in Tables 2 and 3 opposite. These details are extracted from the post mortem findings (Appendix (a), page 92). Despite the lack of immunosuppression, only 3 of the 20 allografts died of rejection. In these 3 animals rejection occurred at a time when it would have been expected in an unprotected animal with an allograft (ie. 5 - 10 days). In the remaining allografts, however, rejection did not appear to play a significant role in causing death. The major cause of death in the combined allo- and autograft groups was gastric ulceration, which was responsible for 40 per cent of the mortality. The second most important cause was sepsis, which usually took the form of a primary bilateral pneumonia or cholangitis. The mean survival times were:

Allografts : 21 days
- Group 1a, 22.2 days
- Group 1b, 19.9 days

Autografts : 23.9 days
- Group 2a, 22.2 days
- Group 2b, 25.7 days

Animals 41 - 43 (cholecystduodenostomy alone controls) gained weight rapidly, appeared normal and were sacrificed at 3 months. Biochemical and haematological values pre-operatively, and at 1, 2 and 13 weeks postoperatively, were normal. At autopsy the liver was normal.

2. CLINICAL OBSERVATIONS

All animals survived 5 days or more. The majority looked well and were ambulant during the first week. During the second week ulcer-haemorrhage or perforation occurred in 40 per cent of the animals. The majority of animals that survived into and beyond the third week showed signs of chronic ill health, with muscle wasting (first shown by prominence of the spine) and a roughened coat. This appearance was reminiscent of a heavily immunosuppressed animal. The majority of animals progressively lost weight, with mean losses (from the preoperative level) of 4 lbs. in the first week, 8 lbs. in the second, 11 lbs. in the third and 12 lbs. at the end of the month. After a month those animals without evidence of infection or chronic rejection tended to gain weight (eg. animals 22, 40), but, where these complications were present, progressive weight loss was the rule. Three autografts were the only animals to gain weight during the first month.

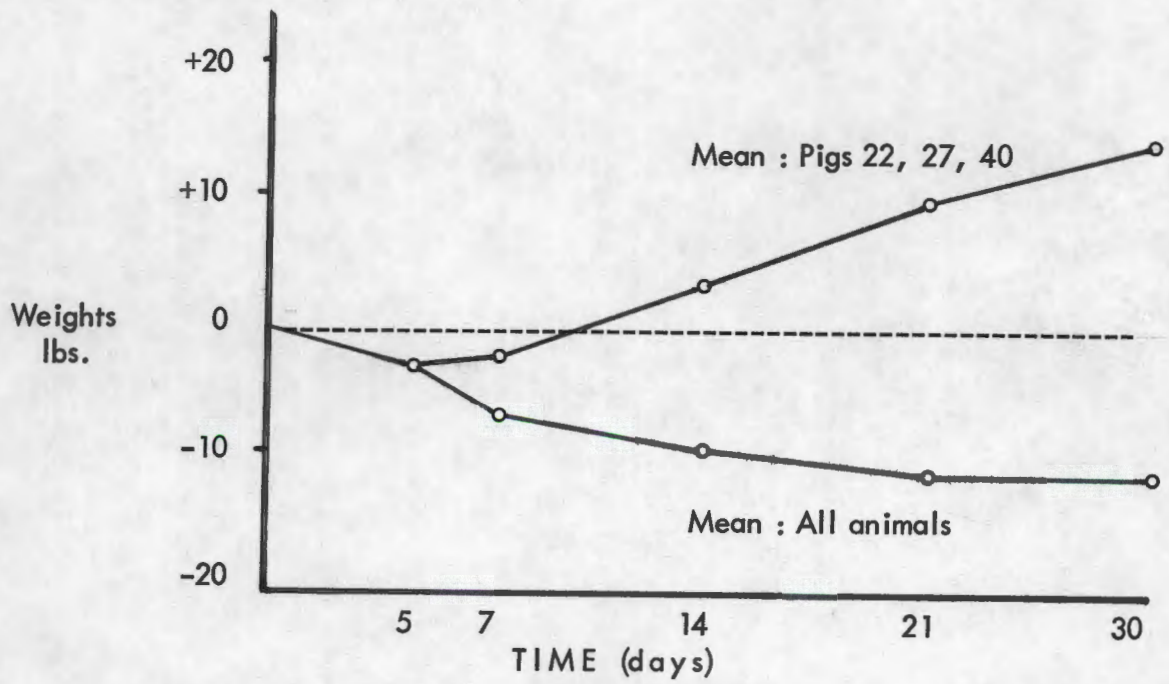


FIGURE 5. POSTOPERATIVE WEIGHTS

3. BIOCHEMISTRY

1. NORMAL RANGE

In 50 animals blood was drawn as the first operative procedure from the subclavian vein in the anaesthetised pig. The mean values and ranges were analysed (79, 179) and are presented in Table 4 with a comparison of results from standard texts.

TABLE 4

Biochemistry	Pigs-this series	Pigs- Loveday*	Human
No:	50 animals	4 animals 36 estimations	varying numbers
Total protein g.%	5.4 (3.9 - 7.4)	5.3 (4.7 - 7.0)	7.9 - 10.3
Albumin g.%	2.1 (0.8 - 3.9)	1.4 (0.9 - 1.9)	2.1 - 4.6
Globulin g.%	3.4 (1.4 - 5.1)	4.3 (1.8 - 5.9)	3.9 - 5.6
Alkaline phosphatase (Shinowara-Jones-Reinhart units)	5.4 (1.2 - 12.1)		2.0 - 4.5 Bodansky units
Aspartate transaminase	42.3 (15 - 140) Karmen units	54.6 (32 - 92) King units	10 - 40 Karmen units
Cholesterol mg.%	89.4 (64 - 144)		150 - 280

These results are included with permission from the Editor, S. African Medical Journal.
* Loveday - personal communication.

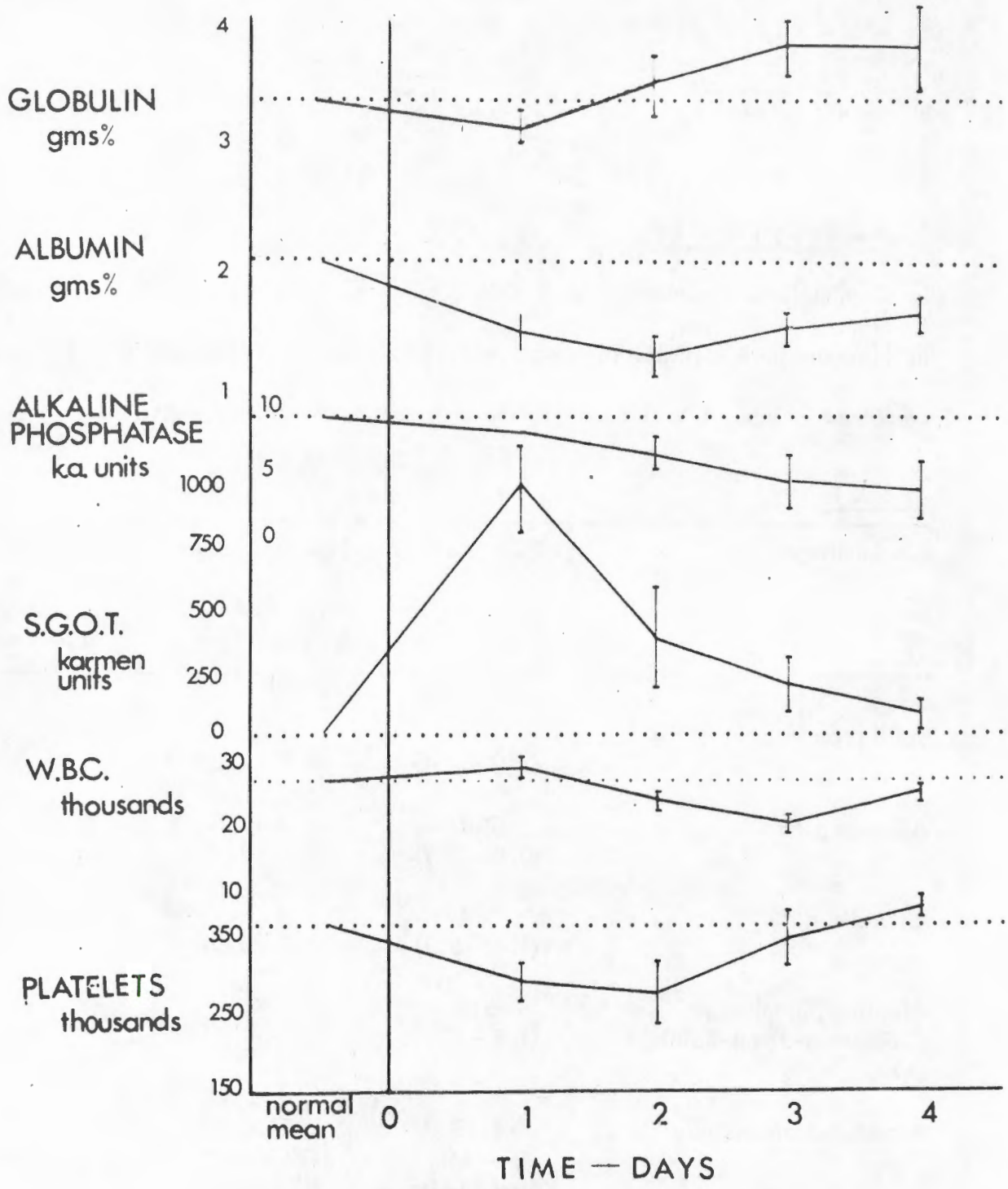


FIGURE 6:

MEAN VALUES & RANGE — DAYS 1—4 (ALL GROUPS)

2. FIRST FOUR POSTOPERATIVE DAYS

The readings in the allograft and autograft series were found to be similar. Detailed results are presented in Appendix (a), page 92. The mean values and ranges in all 40 animals are shown in Fig. 6.

S.G.O.T. A maximal peak of this enzyme was reached at 24 hours, with a mean level of 964 Karmen units. This had returned to near normal by the fourth day.

Alkaline phosphatase There was a steady decline to half the laboratory control levels in all groups.

Albumin The albumin levels fell below the control levels and had not recovered by the fourth day.

Globulin After a transient fall on the first postoperative day, the globulin levels rose progressively.

Bilirubin (i) Autograft group: Two animals (numbers 21 and 25) were jaundiced throughout this period (average levels 2.4 mg.% total and 2.0 mg.% conjugated bilirubin). The remaining 18 autografts were not jaundiced during this period.

(ii) Allograft group: Over half the allografts showed mild jaundice during this period, with total bilirubin levels of never more than 2 mg.%.

Comment: The biochemical disturbance seen during this period was most likely due to the trauma of ischaemia, perfusion and operation. Rejection probably played no part as, not only did the histological changes of rejection only become apparent after the third or fourth day, but also the changes were similar in the allograft and autograft control groups.

Stuart et al. found in dogs that the S.G.O.T. peaked at 24 hours, but returned to normal at the third day (168). Starzl's group had similar findings in this animal (162).

Enzyme release, reflected by the S.G.O.T. levels, is a usual consequence of liver trauma. The fall in alkaline phosphatase levels, however, is not a frequent feature of other experimental liver transplant literature where elevations or normality are described. In one of Terblanche's pigs (175), however, the alkaline phosphatase fell below normal laboratory levels and, in Starzl's human series, it was also noted occasionally (163). Stuart and Moore found immediate postoperative elevations of alkaline phosphatase "only rarely" (168). It is probable that the decline in alkaline phosphatase (and albumin) is a reflection of an impaired protein synthesis by the liver. In the dog post-transplant hypoalbuminaemia has been noted, together with an increase in globulin, identified as the $\alpha 2$ fraction (90). Protein synthesis rate in a small group of dog allografts was shown to decrease postoperatively, but to return to normal by the third or fourth post-transplant day (91). Calne reported reduction in serum albumin in 10 pigs, surviving at 13 - 99 days (31).

The progressive rise in serum globulin could be interpreted in the allografts as the response of the reticulo-endothelial system to the foreign liver; that a similar increase in globulin occurred in the autografts might indicate another mechanism. There was, however, no overt evidence of infection at this time. There would appear to be no entirely satisfactory explanation for the difference in incidence of jaundice between the allograft and autograft groups. Perhaps the most likely one is the fact that the allografted liver

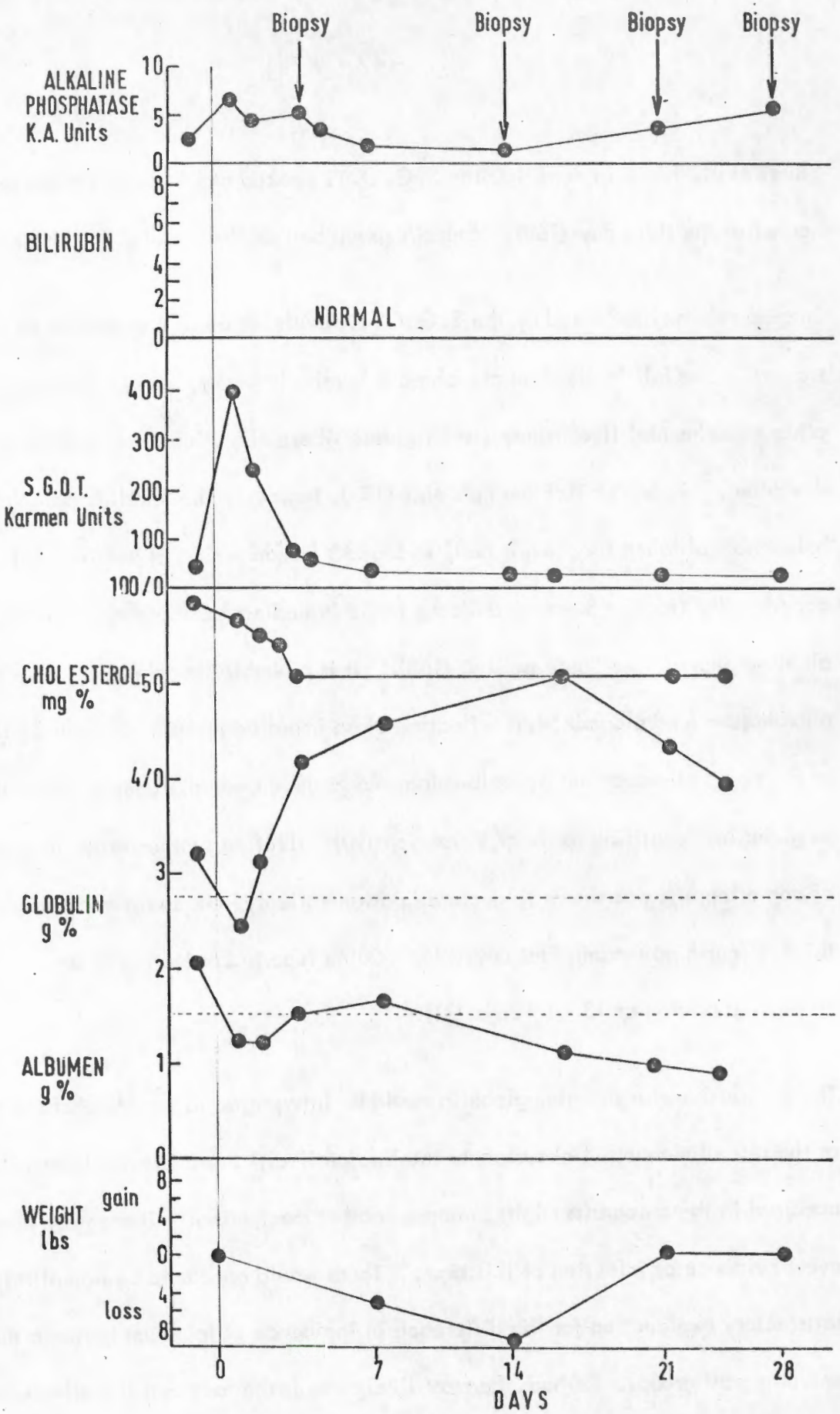


FIGURE 7: Autograft profile (Animal 31)

was submitted to a greater degree of technical manipulation - physical handling, initial portal followed by arterial revascularisation, slower rewarming etc. The ischaemic times, perfusates and blood transfusions were comparable in both groups.

3. SUBSEQUENT COURSE OF THE AUTOGRAFT GROUPS

(i) Group 2b (Choledochodochostomy autografts) showed no further abnormalities of bilirubin or S.G.O.T. after the fifth day. There was, however, a progressive decrease in serum albumin to levels below the lowest normal range for the laboratory for periods of up to a month. In addition, the alkaline phosphatase levels remained subnormal for up to 2 weeks. (Fig. 7).

Comment: The absence of rejection, necrosis and biliary stasis in this group (as shown on biopsy and by the normal S.G.O.T. and bilirubin levels) highlighted an anicteric liver dysfunction without enzyme release. This must have been a result of the trauma of ischaemia, perfusion and operation and is obscured in the other groups by changes due to rejection and biliary stasis.

(ii) Group 2a (Cholecystduodenostomy autografts), on the other hand, showed early evidence of biliary stasis. S.G.O.T. and alkaline phosphatase readings were on all occasions higher than in Group 2b (Table 5). These biochemical observations, indicative of cholestasis, were supported by histological evidence in simultaneous biopsies. The albumin levels were similar to Group 2b.

Biochemical Measurement	First Week				Second Week			
	Allograft		*Autograft*		*Allograft*		*Autograft*	
	1*a*	1*b*	2*a*	2*b*	1*a*	1*b*	2*a*	2*b*
SGOT (Karmen units)	146	122	156	32	93	89	98	54
Conjugated bilirubin (mg. per cent)	2·8	1·8	0·4	<0·5	1·4	0·5	0·6	<0·5
Alkaline phosphatase (K.A. units)	4·9	6·8	4·4	2·2	5·5	7·7	3·9	2·3
No. of observations	9	9	7	10	5	7	5	10

TABLE 5 Mean biochemical values for each group at 1 and 2 weeks, showing greater deviation from normal in the allografts when compared to the autografts, and when cholecystduodenostomy was used compared to choledochodochostomy.

(With permission of the Editor of the British Journal of Surgery)

Comment: These findings suggested that cholecystduodenostomy caused biliary obstruction, as evidenced by the biochemical signs of cholestasis.

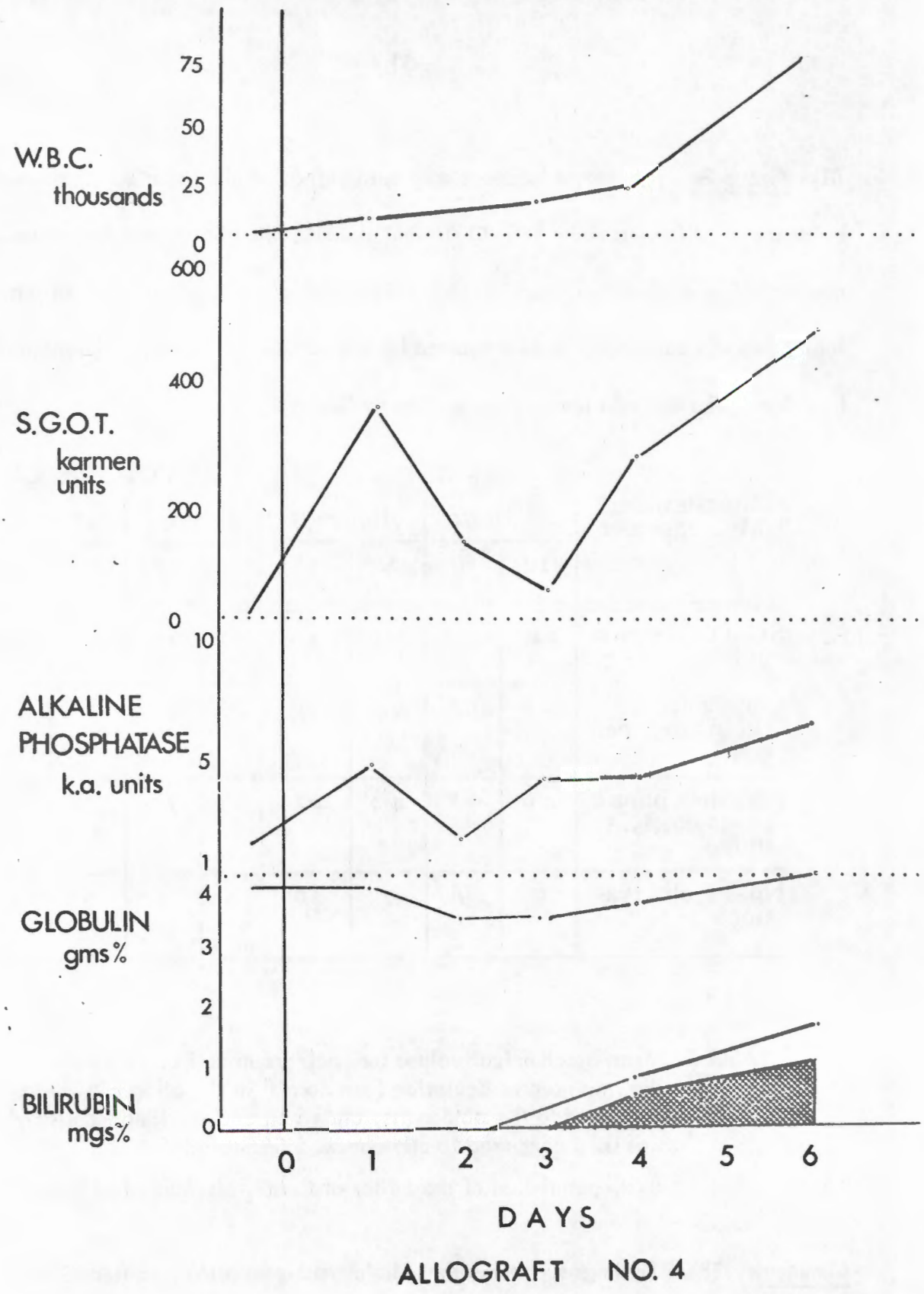


FIGURE 8: Fatal rejection

4. SUBSEQUENT COURSE OF THE ALLOGRAFT GROUPS (1a, 1b)

(i) Compound biochemistry

At the end of the first and second weeks the mean levels of S.G.O.T., alkaline phosphatase and cholesterol were higher than in the corresponding autograft controls (2a, 2b), (Table 5, page 51). There was also a greater incidence of jaundice in the allograft groups.

Comment: During the first 5 days the postoperative jaundice seen in most of the allografts, and not the autografts, was attributed to a greater degree of physical trauma. There was no difference at this time in S.G.O.T. or alkaline phosphatase. The later differences in these modalities, however, was probably due to the rejection process, as all biopsies at this time showed some signs of rejection.

(ii) Acute fatal rejection

Three animals (2, 4 and 16) died on the fifth, sixth and seventh postoperative days respectively, with histological changes of severe rejection. The biochemical profile of these animals (Fig. 8) showed initial partial recovery from the operation, followed by S.G.O.T. release and progressive jaundice, although the alkaline phosphatase did not always rise significantly.

Comment: These changes are reminiscent of those described in the unimmunosuppressed dog (160, 111), and also sporadically in th pig literature (175, 77, 166, 25, 106).

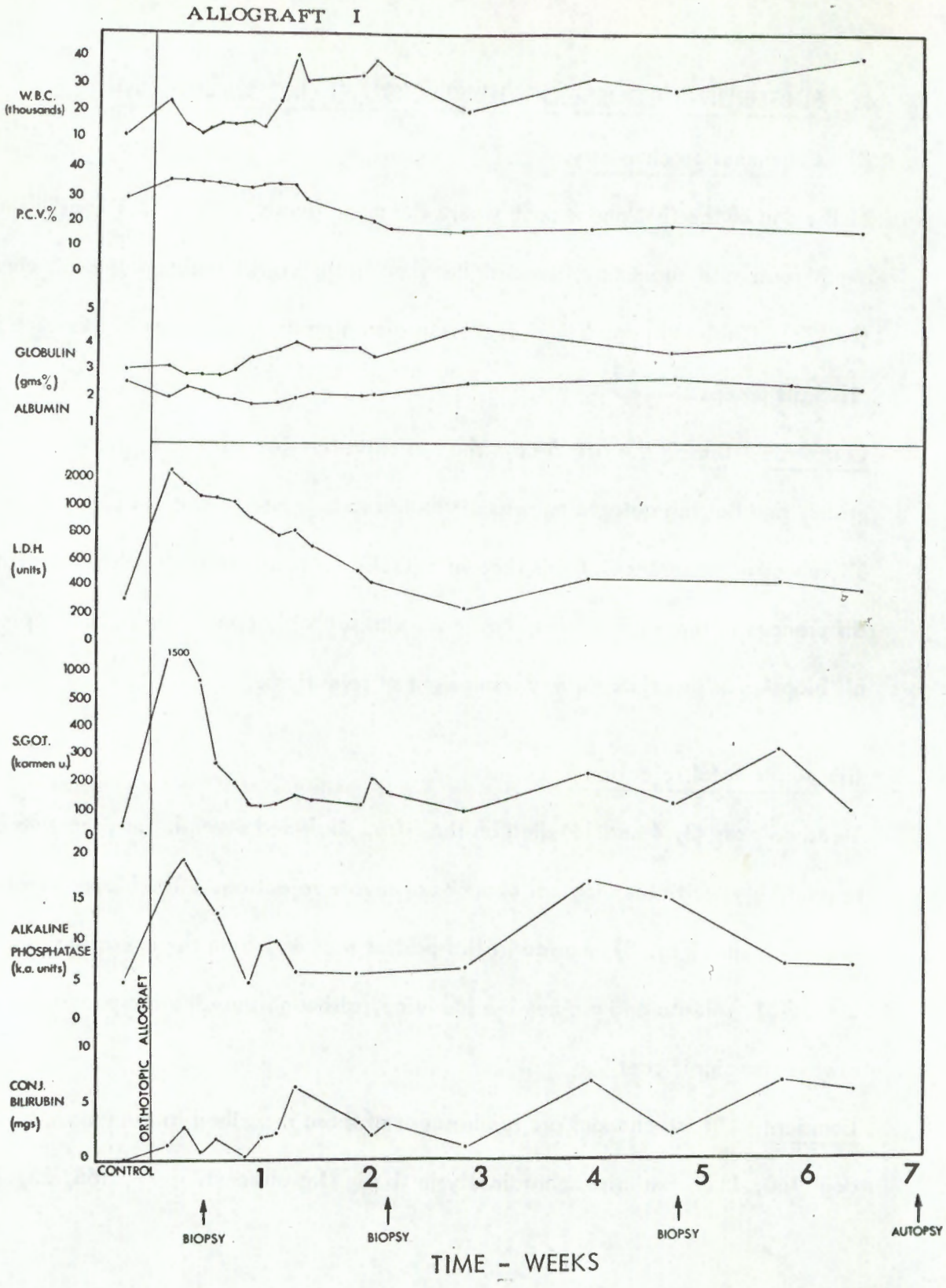


FIGURE 9: Low grade rejection

(iii) Chronic rejection

The remainder of the allografts showed low grade rejection as evidenced by moderate round cell infiltration seen on the biopsies. The biochemical profiles of these animals ran a fluctuating course (Fig. 9). In all animals the S.G.O.T. showed evanescent peaks, but the alkaline phosphatase and bilirubin were usually only elevated when there was evidence of severe biliary stasis or cellular necrosis. Frequently, biopsies taken during periods of maximal biochemical change revealed only biliary stasis with minimal evidence of rejection.

Comment: Elevation of alkaline phosphatase, bilirubin and, to a lesser extent, S.G.O.T. are regarded as biochemical indications of liver rejection in all experimental animals (152, 162, 168, 175, 106, 77, 32). The fluctuating and spontaneously reversible changes seen are similar to those reported by Garnier (77). It is true to say that, in this series, these modalities were nearly always elevated when rejection was present; it is, however, also true to say that an identical pattern of elevation was seen when simple cholestasis was present (as, for example, in the autograft control Group 2a). It appeared that cholestasis could be a manifestation of either rejection or biliary obstruction and that the biochemical profile was identical. The difficulty of diagnosing rejection on these biochemical criteria alone is apparent.

4. HAEMATOLOGY

The haematology was limited to estimations of the haemoglobin, packed cell volume (pcv), white cell count (wbc) and platelet count. Observations were made daily for a week and then at approximately weekly intervals.

1. NORMAL RANGE

Blood was drawn from the subclavian vein catheter soon after commencement of anaesthesia in 46 animals, which had been maintained on glucose water for 12 hours preoperatively. The mean values and ranges were analysed and are presented below.

TABLE 6

	Pigs - this series 46 animals	Pigs - Blecher (11) varying numbers	Human varying numbers
Haematocrit %	31.3 (20 - 50)	33.7 (28 - 42)	42 - 47
Platelets x 1000 mm^3	360 (144 - 530)	407 (220 - 665)	200 - 500
Leucocytes x 1000 mm^3	17 (6 - 30)	19 (11 - 33)	9 (4 - 11)

2. FIRST FOUR POSTOPERATIVE DAYS

The mean values of white cells and platelets are indicated in Fig. 6, page 48. The values for the allograft groups (1a, 1b) were found to be similar to the autograft groups (2a, 2b). Both were depressed during this period but returned to normal by the fourth day. The pcv usually showed a 5 - 10 per cent increase on the first post-operative day, to return to normal over the subsequent days.

Comment: The fact that depression of the white cell and platelet counts occurred in both allografts and autografts suggests that immunity played no part in the process. The volumes of blood transfused are given in Table 1, page 38, and could have contributed to the leucopaenia and thrombocytopaenia. It is also possible that the cells could have been sequestrated in the traumatised tissue. Mowbray (100) has suggested that thrombocytopaenia is an early sign of rejection, the platelets becoming sequestrated on the vascular endothelium of the graft. Postoperative thrombocytopaenia has also been noted by Starzl in a series of pig allografts (166) and by Hutchinson in dogs (83). The results of the present controlled series, however, would suggest that platelet depression is a result of ischaemia and perfusion, rather than the earliest changes of rejection.

3. SUBSEQUENT PATTERNS

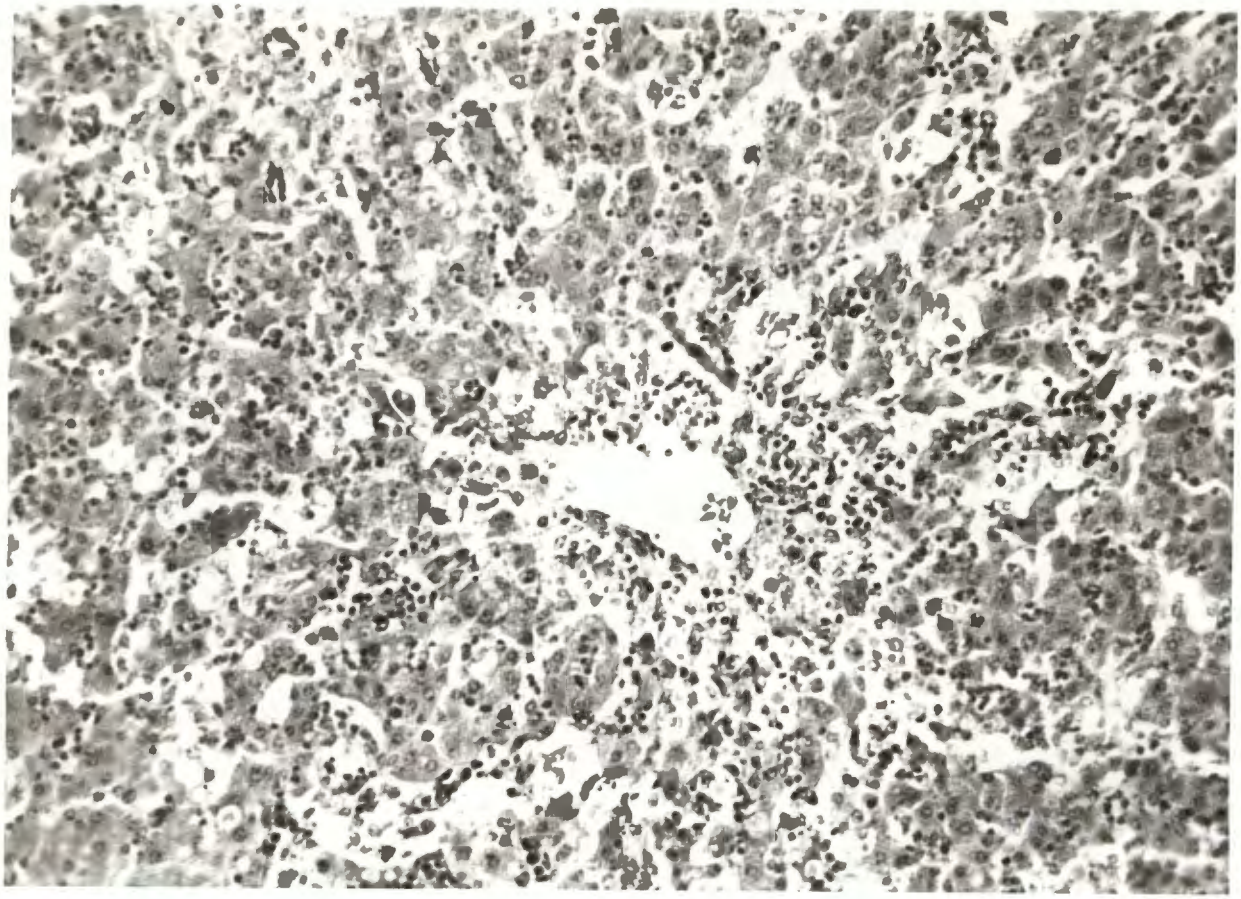
(a) Acute fatal rejection: (Fig. 8, page 52) The white cell count became elevated in all 3 cases to levels of 50 - 75 x 10^3 cells/mm^3. The platelet counts were inconsistent in that mild elevation or depression were seen.

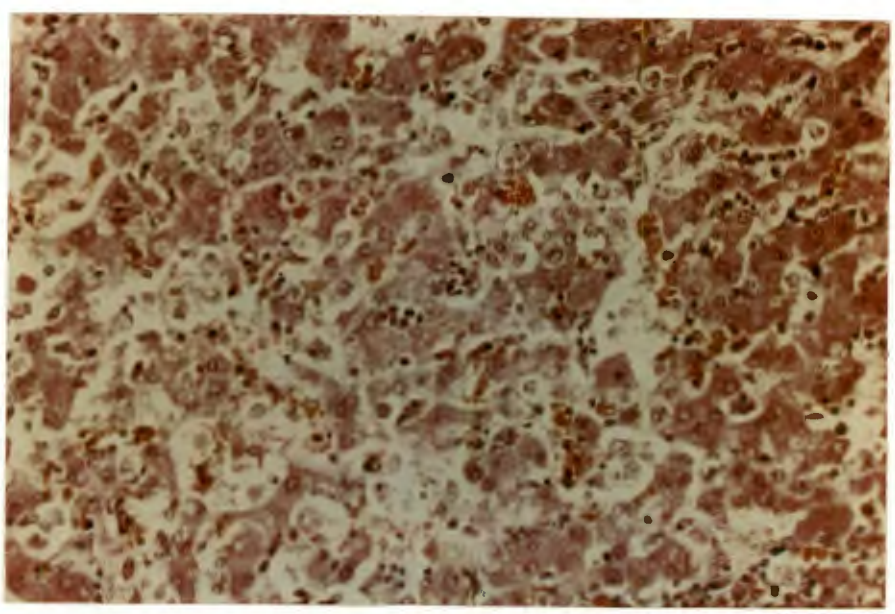
(b) Gastro-intestinal haemorrhage: (Fig. 20, page 79) There was a progressive fall in the pcv. The white cell and platelet counts were variable.

(c) Chronic rejection: (Fig. 9, page 53) The white cell count became elevated and fluctuated widely between 20 - 40 x 10^3 cells/mm^3, when there was biopsy evidence of rejection. In many animals this observation was distorted by the presence of cholangitis or other infection.

(d) Infection: Infection was invariably accompanied by leucocytosis.

(e) Normality: Normality was found in Group 2b before the development of pneumonia.





FIGURES 10 and 11: Allograft 4. Histology of acute fatal rejection. There is dense round cell infiltration of the hepatic parenchyma, haemorrhage, cellular dissolution and necrosis. (Haematoxylin and eosin x 57).

(Figure 10 by permission of The Editor, The British Journal of Surgery).

5. HISTOLOGY

1. ALTERATIONS IN THE FIRST FOUR DAYS

There was Kupfer cell swelling and minimal polymorphonuclear cell infiltration - changes that had disappeared by the fifth day.

Comment: This histological picture, similar in all groups, reflected the trauma of ischaemia and perfusion and, by its subdued nature, contrasted strongly with the biochemical disturbance seen at this time (Fig. 6, page 48). These histological changes are similar to those seen in dog livers by Porter (126), and in dog liver autografts (99).

2. FATAL ACUTE REJECTION

Fatal, acute rejection was found in 3 animals (pigs 2, 4 and 16) dying on the fifth, sixth and seventh postoperative days respectively. The histology in each case was similar, with all the hallmarks of an acute immunological attack (Figs. 10 and 11).

Comment: This histological picture is similar to that seen in the majority of unimmunosuppressed dogs, as reviewed by Porter (126), and in a human liver transplant, treated without immunosuppression, as reported by Williams et al. (185). In pig allografts there is no general agreement on the incidence of rejection, as shown in Table 10, overleaf. Two pigs described by Hunt (81) died from rejection at 8 and 10 days with less vivid histology, and four pigs had histological rejection in Mieny's series (106). Calne, however, described advanced hepatic rejection only in pigs previously sensitised by renal allografts and had never encountered this degree of rejection in a conventional

TABLE 10

UNIMMUNOSUPPRESSED ANIMALS LIVING MORE THAN 5 DAYS
CALCULATED PRIMARY CAUSES OF DEATH IN VARIOUS SERIES

	PRESENT SERIES	BELZER	CALNE	GARNIER	MIENY	STARZL	TERBLANCHE
FATAL REJECTION	3	8	0	4	4	4 (2)	2
ULCERATION	12	2	3	0	0	(2)	6
INFECTION	3	0	1	0	1	0	0
OTHER/UNKNOWN/ UNPUBLISHED	2	0	22	3	2	3	4
TOTAL	20	10*	26**	7	7	7***	12
Reference:		(25)	(29,31,39)	(77)	(106)	(166)	(175)

* 5 stored 8 - 10 hours
** majority vagotomised
*** 2 animals had both gastro-intestinal haemorrhage and rejection

allograft (29, 31, 39). Porter reviewed a Denver series of porcine hepatic allografts and found in 4 animals, dying at 8, 8, 20 and 37 days, that the rejection was "much milder ..... than in the untreated dog" (126). There were 3 cases of lethal rejection in Garnier's series of 7 pigs (77) and Belzer's group (25) found 8 of 10 pigs (5 with livers stored for 8 - 10 hours) died from rejection. Possible reasons for these qualitative and quantitative differences between various allograft series will be discussed later.

3. LOW GRADE REJECTION

Low grade rejection occurred in the majority of animals (Table 2, page 45). There was moderate round cell infiltration which was maximal during the second week (Fig. 12).

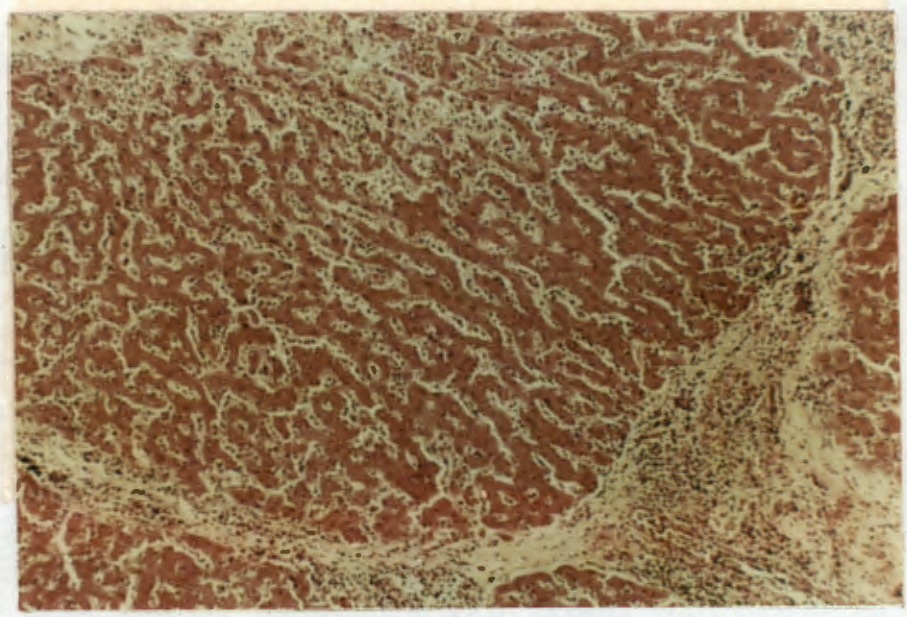


FIGURE 12: Allograft 14. Second week: There is moderate round cell infiltration around portal tracts, sinuses and throughout the lobule (Haematoxylin and eosin x 57).

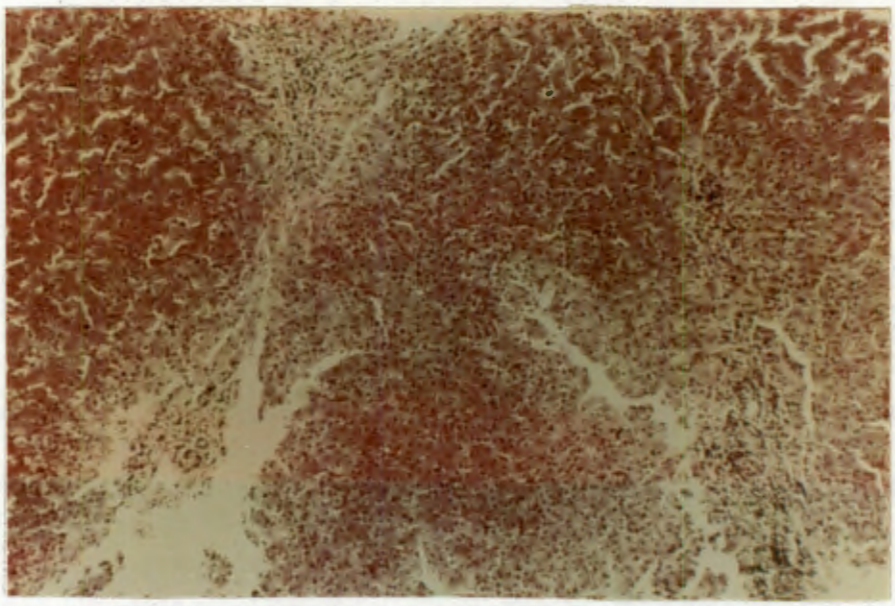


FIGURE 13: In vivo biopsy of pig 19 at 8 days. Moderately severe round cell infiltration is present. (Haematoxylin and eosin x 57).

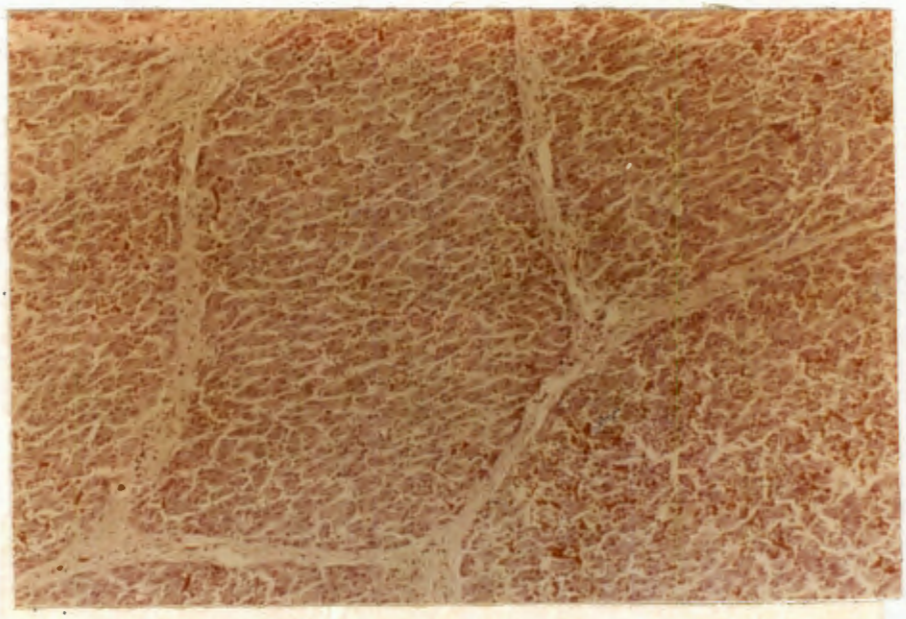


FIGURE 14: Autopsy of same animal (pig 19) at 38 days. Representative section showing minimal evidence of rejection. (Haematoxylin and eosin x 57).

When the in vivo biopsies were examined serially, the degree of infiltration was shown to diminish in several animals over subsequent weeks (Fig. 13). This phenomenon would have been missed had only autopsy material been examined (Fig. 14). Vascular changes were seen on occasion in long-living animals, but never in those dying in less than 10 days.

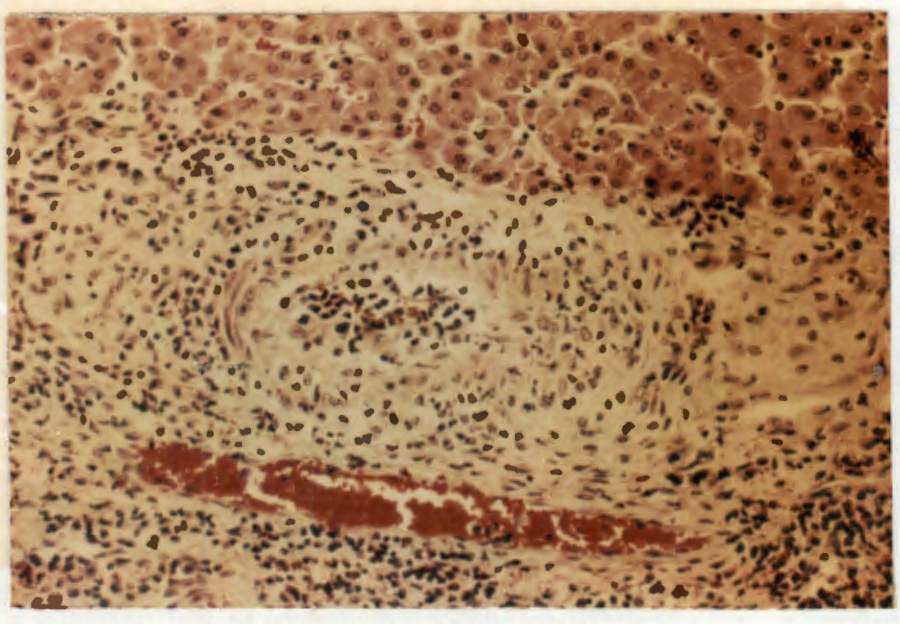


FIGURE 15: Histological changes of vascular rejection, showing round cell infiltration surrounding the arteriole. (Haematoxylin and eosin x 114).

Comment: These histological changes are similar to those reported by most groups in a proportion of their animals (29, 126, 77, 81). The spontaneous diminution of round cell infiltration has previously been noted by various workers in pigs (175, 77, 166) and in dogs (154, 111).

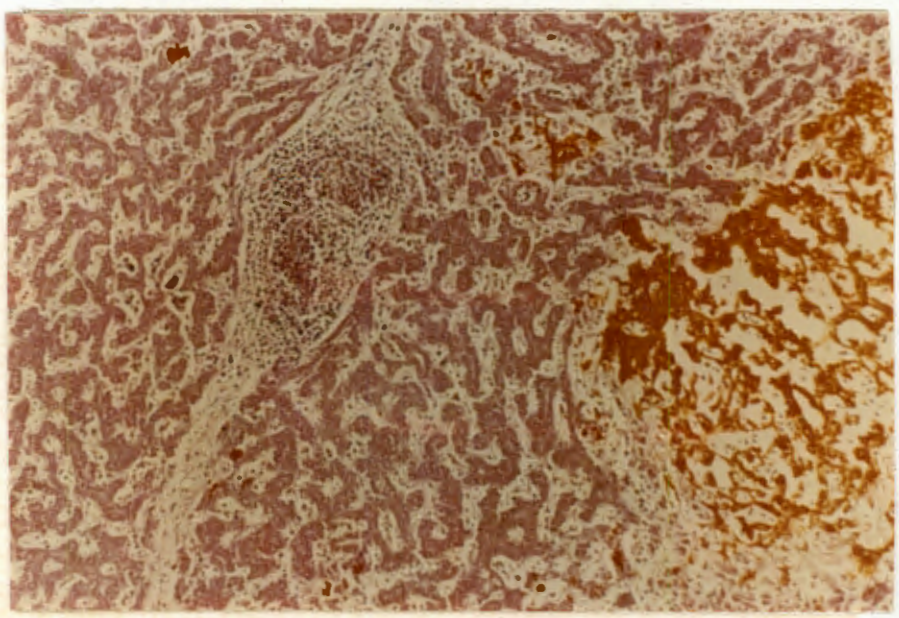


FIGURE 16: Allograft 1. Bile plugs and a bile lake - histological evidence of cholestasis. Cholangitis is also present. (Haematoxylin and eosin x 57).

(With permission of The Editor, The British Journal of Surgery)

4. BILIARY STASIS

(i) Biliary stasis was seen as a frequent concomitant of the rejection process and this association was in agreement with the biochemical observations made previously. Cholestasis manifested as bile plugs or even bile lakes (Fig. 16).

(ii) Biliary stasis was frequently accompanied by cholangitis in the groups where the gall bladder was anastomosed to the duodenum (Groups 1a, 2a) (Fig. 17).

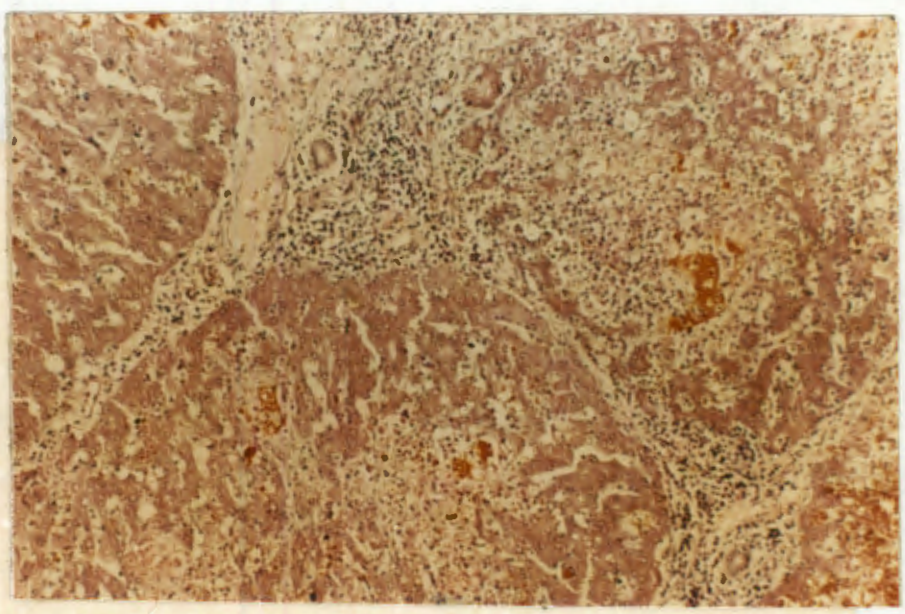


FIGURE 17: Pig 1. Biliary stasis, cholangitis and rejection; a frequent pathological triad in Group 1a. (Haematoxylin and eosin, x 57)

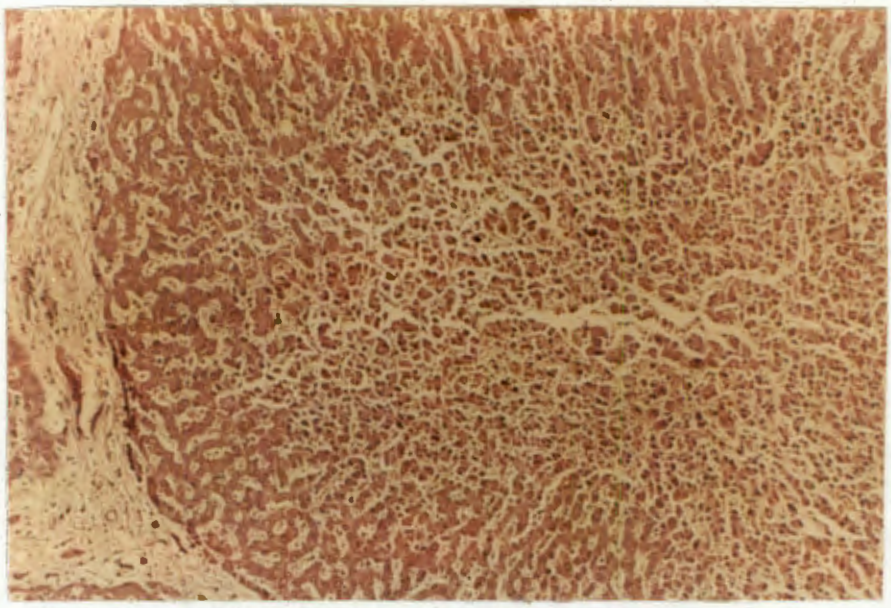


FIGURE 18: Pig 27. Centrilobular zonal necrosis, showing the eosinophilic area of dead hepatocytes in the centre of the lobule. (Haematoxylin and eosin x 57).

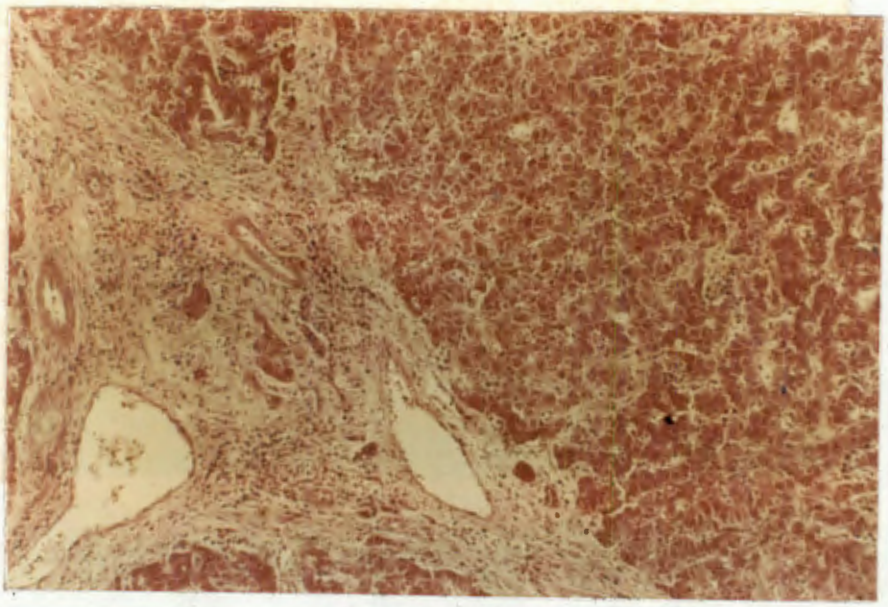


FIGURE 19: Pig 22. Commencing interlobular fibrosis at 70 days. (Haematoxylin and eosin x 57).

5. CENTRILOBULAR ZONAL NECROSIS (Fig. 18)

This change was found sporadically in all groups and was usually associated with a protracted death from pneumonia or haemorrhage. The pathogenesis was thought to be one of poor hepatic perfusion associated with agonal shock, as it was seen in the autografts which died in this way. The biochemical changes and their significance are discussed on page 69. Moore's contention (111) that rejection is a primary vascular phenomenon was not supported by this observation, or indeed by the fact that vascular rejection was an uncommon finding. Porter found vascular changes in only 25 per cent of the dog livers he reviewed (126).

6. FIBROSIS (Fig. 19)

Fibrosis was seen in both long-living autografts and allografts. This change was seen mainly around the portal tracts, but also extended into the lobules on occasions.

Comment: Fibrosis has been reported in long term survivors of hepatic transplantation. Porter reported it in Starzl's long surviving humans (126), Starzl reported this in the dog (160) and its presence has been noted frequently in the pig (31, 77). Terblanche, moreover, found that fibrosis increased in a single animal studied over a year (175). The presence of fibrosis is not invariable in long term survivors of liver transplantation, but, when it does occur, appears progressive and lethal. Porter reported on Starzl's 2 longest surviving dogs (126): "The cellular infiltration diminished over the ensuing 443 and 1041 days respectively, but the fibrosis progressed to severe cirrhosis and eventually killed the animals". He also noted in one of Starzl's human transplants:

"In one homograft (O T 11) the hepatic fibrosis had progressed to cirrhosis, with regeneration nodules and foci of hepatocyte necrosis".

The fact that fibrosis was noted in the control groups would indicate that this change is a non specific response to liver damage in this species. Further support for this contention comes from a similar finding in dogs submitted to simple hepatic ischaemia for periods of over an hour (171).

DISCUSSION

It has been a controversial point whether rejection of the pig liver allograft differs quantitatively and qualitatively from other species.

It is suggested in man that rejection occurs in the "expected" fashion (ie. fatal liver rejection in the majority of cases in 5 - 10 days, with a lethal assault on the graft by the host). This evidence comes from a report of a human who received a liver transplant without immunosuppression and who died from fatal rejection (185). The suggestion also arises from circumstantial evidence in the treated cases (166, 39). Extensive research shows that both the dog (156, 111) and the rat (93) undergo a similar form of rejection, as does the baboon (119) and the rhesus monkey (39). It would appear that in all these species rejection occurred in the "expected" fashion. It would also appear that in these species there was no great immunological preference or difference between kidney, heart and liver. Is the pig, therefore, "privileged" or "favoured" (175, 129), and does the porcine liver have an "immunosuppressive effect"? (29)

It is known that the pig rejects allografted kidneys normally (31, 68), as it does skin (8, 87). It is also suggested that the heart is "normally" rejected (39). Further evidence for the normal immunological competence of the pig comes from lymphocyte studies: there is a normal lymphocyte transfer reaction (87) and a typical mixed lymphocyte reaction. It appears, therefore, that the postulate is more specifically related to a property of a single organ - the liver. Alternatively, the postulate could refer to the response of this species to this particular organ.

Typical fatal rejection was found in 3 allografts (15 per cent) in this series. The primary causes of death of all the major immunosuppressed porcine series are analysed in Table 10, page 58. Of the 89 published pig liver transplants, 25 died from lethal rejection. The 3 examples in the present study were typical of a host versus graft reaction. There is no doubt that fatal rejection does occur in this animal and no support is found for Calne's contention that it does not (39).

The incidence of fatal rejection varies from 15 per cent (in the present study) to 80 per cent (25), with an average of 40 per cent when Calne's series is excluded.

Many animals, however, both in the present series and in those with which it is compared, lived for prolonged periods without immunosuppression. In addition, the liver histology of this type of animal, both in the present series and others (39, 175, 77), showed an essentially mild rejection response. It would appear, therefore, that a proportion of pigs undergo a mild rejection process and may live for prolonged periods

without immunosuppression. Histocompatibility must influence the nature of rejection. It is perhaps no coincidence that in this series all 3 cases of acute fatal rejection took place in cross breed experiments and that the 2 longest survivors were littermates. Perhaps the long survival of Calne's pigs (31), and Terblanche's long term survivor (175), could be attributed to the histocompatibility of littermates or related transplantation. However, mild rejection was also seen when transplantation in the present series was undertaken across the histocompatibility barrier of different breeds.

The author's view would coincide with that of Starzl - that the liver allograft response in the pig differs only quantitatively from other animals. Starzl computed that the survival time for the unimmunosuppressed dog is 7.1 days (160); the mean survival time for the untreated pig in this series was 21 days. Starzl found that some dogs could exceed this period on occasion by some months (and he attributed this to change in compatible match); in pigs a greater proportion are able to do this. It would appear to be a quantitative phenomenon that rejection occurs in a milder form in a greater proportion of animals. There is certainly little evidence that the porcine liver is "qualitatively unique".

6. BACTERIOLOGY

Infection is a well documented complication of liver transplantation. In humans infection has been responsible for considerable mortality and is fully reviewed by Fulginiti (65) and Starzl (166). Hepatic abscesses have been frequently reported after dog liver transplantation (59, 160, 168), the infection occurring despite antibiotic cover. In pigs hepatic infection was a marked feature of Calne's animals surviving more than 4 days (31), but not in the series of Terblanche et al. (175), or Mieny et al. (106). Five pigs, sacrificed after transplantation at 5 - 7 days, were all found to have hepatic or perihepatic abscesses by McSween (116), and the bacteriological cultures were discussed by Brettschneider et al. (19).

RESULTS

(i) The duodenal content of 5 normal pigs was cultured to determine the normal duodenal flora. The results are presented in Table 7, below.

TABLE 7

		COLIFORMS	YEASTS	DIPTHEROIDS
ANIMAL	a	+	+	
	b		+ (candida)	
	c			
	d		+ (not candida)	+
	e	+		

TABLE 8 BACTERIOLOGY

GROUP	PIG	SURVIVAL (days)	PRIMARY CAUSE OF DEATH	SOURCE OF CULTURE	CHOLANGITIS	E. Coli	Proteus	Pseudomonas	Enterococci	Bacteriodes	C. Welchii	Klebsiella	S. Typhimurium	Staph.	B. Strep
1a	1	51	Generalised infection	Wound abscesses		+	+	+	+		+			+	
1a	5	21	Pneumonia	Liver, lung	+	+				+			+		
1b	11	13	Generalised infection	Liver, blood	+						+				
2a	21	17	Generalised infection	Liver, lung	+						+				
	27	35	Pneumonia		+										
	29	27	Generalised infection	Lung	+				+					+	
	30	28	Generalised infection	Liver, wound		+			+						
2b	31	28	Pneumonia	Lung			+	+							
	32	24	Pneumonia	Lung			+	+							+
	33	17	Pneumonia	Lung		+	+								+
	35	21	Pneumonia	Lung, liver		+	+								
	37	18	Pneumonia	Lung				+							
	38	17	Pneumonia	Lung		+									
	39	22	Pneumonia	Lung			+		+			+			

(ii) In the present series 14 animals died from infection. The causes of death and the organisms responsible are presented in Table 8. The majority of infections were due to multiple organisms, gram negative combinations being the most common.

Pneumonia was the primary cause of death in 9 animals, occurring between 17 and 35 days. The lungs were usually bilaterally involved with confluent consolidation. Pulmonary abscesses and pleural effusions were frequently found. The organisms were usually combinations of E. Coli, Proteus, Pseudomonas or Enterococcus.

Generalised infection was found in 5 animals, with a positive blood culture and generalised pyaemic abscesses in lungs, liver, subcutaneous tissues and various viscera. Death in this manner occurred at 13, 17, 27, 28 and 51 days.

Four animals succumbed from infection by a single organism - E. Coli, Pseudomonas and Cl. Welchii in 2 animals.

DISCUSSION

The incidence and type of infection varied within the four experimental groups. Only 3 of 20 animals died from infection in the allograft groups 1a and 1b; on the other hand, 11 of 20 animals succumbed from infection in the autograft control groups 2a and 2b. In the allograft groups with a low incidence of infection, early gastric ulceration took a toll; in the autograft control groups, where gastric ulceration was minimal, late infection took a toll. It is therefore apparent from these figures that the vast majority

of animals that did not succumb to the early complications of gastric ulceration, died subsequently of late infection.

Pulmonary complications are common after liver transplantation. Starzl (160) found that 46 of 69 dogs, living longer than a week, had pneumonitis or atelectasis. Infectious pulmonary complications were also common in his human series (166).

That 14 animals should succumb from sepsis, out of an experimental group of 40, implies extensive infection. This degree of infection was not encountered in the same laboratory after other experimental procedures (cholecystduodenostomy, gastric operations, renal allografts), and implies that the liver autograft and allograft procedures put the animal at a special disadvantage. That the infection usually only became apparent 2 or 3 weeks after the animal had been in the sty, suggests a reduction in host resistance postoperatively, rather than intraoperative infection.

The organisms encountered have been shown by Brettschneider et al. (19) to be the normal commensals of the pig gastrointestinal tract; the fact that they became invasively pathogenic at the second or third week is further suggestive of impaired host resistance.

Ascending infection of the bile duct could provide one portal of systemic entry. In this series cholangitis was dramatically reduced when choledochodochostomy was employed instead of cholecystduodenostomy, as will be seen in Section 7, Chapter IV. This supports the observations of other groups (4, 19, 116). Yet this route of access

could not account for all the cases of infection, as in Group 2b, where cholangitis was eliminated by preservation of the sphincter of Oddi, pneumonia occurred in 7 of 10 animals.

Portal vein bacteraemia is common in the normal dog (24), occurring in half the animals. Bacteria are also present in the portal vein of the normal pig, but occur less frequently (19). That the systemic infections were usually due to bowel commensals, would suggest that this was the major route of invasion.

The liver occupies a strategic position between the bowel and the systemic circulation, and liver damage could easily result in systemic infection, as postulated by Starzl (166). In the present series, unlike that of Starzl, immunosuppression was not employed, and the autograft series removed the additional factor of rejection injury. It therefore becomes apparent that the trauma of ischaemia and perfusion alone are sufficient to inflict an injury such that systemic bacteraemia is possible.

7. CORRELATIONS

1. HISTOLOGY AND BIOCHEMISTRY

The histology of the in vivo needle biopsies was correlated with the biochemical findings from blood drawn simultaneously and various patterns were found to emerge.

(i) Terminal zonal necrosis pattern

When biochemical studies were performed on animals with gastric ulcer haemorrhage or severe infection within 24 hours preceding death, there was on most occasions steep elevation of S.G.O.T., alkaline phosphatase and bilirubin, where previously these had been normal or near normal (Fig. 20, page 79). This picture was found in 3 autografts (numbers 28, 29 and 39) and 4 allografts (numbers 3, 6, 9 and 13). In all these animals at autopsy the histological feature was that of centrilobular zonal necrosis (Fig. 18, page 61).

Comment: Several papers in the literature have laid store on the terminal biochemistry (166, 98, 19). The last biochemical readings, however, probably do not give a true indication of rejection or cholestasis, but possibly represent the terminal change of systemic hypotension and liver ischaemia. They might indeed be misinterpreted as rejection, but this finding in the autograft series emphasises this pitfall.

(ii) Immediate liver damage pattern

In 3 animals (numbers 1, 25 and 21) the histological picture was different, showing only

scattered areas of focal necrosis. In these the elevation of S.G.O.T., alkaline phosphatase and bilirubin was sustained and did not return quickly to normal as in the other groups. These findings are taken to suggest initial, possibly operative, ischaemia rather than ischaemia due to terminal causes.

(iii) Biliary stasis pattern (With or without rejection; with or without cholangitis) Animals (numbers 1, 3, 4, 8, 13, 14, 17, 21, 25, 26 and 39) with histological evidence of bile stasis (Fig. 16, page 60) usually showed elevations of S.G.O.T., alkaline phosphatase and bilirubin. Whatever the aetiology, the biochemical pattern of biliary stasis was usually fluctuant, sometimes lasting for several weeks (Fig. 9, page 53).

(iv) Cholangitis

The histological signs of cholangitis were accompanied by virtual biochemical normality in many animals. Only when biliary stasis was present was there biochemical abnormality.

(v) Rejection

When round cell infiltration of the portal tracts and liver parenchyma was seen in the biopsies, there was almost invariably some concurrent elevation of S.G.O.T., alkaline phosphatase and sometimes bilirubin.

(vi) Normal histology

Many of the choledochodochostomy autograft controls showed no abnormality after the first week. There were, however, signs of defective hepatic function as evidenced by hypoalbuminaemia and low alkaline phosphatase (page 50).

Comment: The importance of needle biopsy in diagnosis was emphasised by the simultaneous comparison of histology and biochemistry. Biochemical abnormality was found to be non specific in that the abnormalities of rejection and biliary obstruction could be identical. There was no characteristic or diagnostic biochemical pattern. It could be argued that if, after liver transplantation, a "cholestatic" biochemical profile became evident at the end of the first week, the cause was probably rejection. Liver biopsy, however, provided the only finite answer.

7. CORRELATIONS

2. CHOLESTASIS, CHOLANGITIS, BILIARY DRAINAGE AND REJECTION

The experiment was designed to test two forms of biliary drainage (choledochodochostomy and cholecystduodenostomy) in the liver transplant animal. To remove any changes purely due to the rejection process, a similar series of autograft controls was prepared under a similar protocol. In this way, by deduction, changes consequent upon the rejection process and the type of biliary drainage could be defined and separated as shown in the statistical model below.

	CHOLECYST-DUODENOSTOMY	CHOLEDOCHO-DOCHOSTOMY	
ALLOGRAFT	10 (1a)	10 (1b)	20
AUTOGRAFT	10 (2a)	10 (2b)	20
	20	20	40

RESULTS

The superiority of one form of biliary drainage and the "cholestatic" manifestations of the rejection process were shown by two groups of evidence.

(i) Biochemical (Table 5)

When the biochemical values for each group were analysed at the end of the first and second weeks, there was evidence of biochemical cholestasis in the allografts, irrespective of the type of biliary drainage employed.

Biochemical Measurement	FIRST WEEK				SECOND WEEK			
	Allograft		*Autograft*		*Allograft*		*Autograft*	
	1*a*	1*b*	2*a*	2*b*	1*a*	1*b*	2*a*	2*b*
SGOT (Karmen units)	146	122	156	32	93	89	98	54
Conjugated bilirubin (mg. per cent)	2·8	1·8	0·4	<0·5	1·4	0·5	0·6	<0·5
Alkaline phosphatase (K.A. units)	4·9	6·8	4·4	2·2	5·5	7·7	3·9	2·3
No. of observations	9	9	7	10	5	7	5	10

TABLE 5 (Repeated from page 51)

The mean levels of S.G.O.T., alkaline phosphatase and bilirubin were higher in the allografts than in the autografts. This indicated that cholestasis was a rejection associated abnormality. In the autograft controls, however, these values were higher in the cholecystduodenostomy series than in the choledochodochostomy series. This suggested that cholecystduodenostomy produced biliary obstruction.

(ii) Autopsy histology (Tables 2 and 3, page 45)

Histological cholestasis was present in high proportion in the allografts, irrespective of the biliary anastomosis. This was regarded as further evidence that cholestasis was a histological concommitant of the rejection process. Cholestasis was found in 70 per cent of the autograft control series with cholecystduodenostomy, but in only 20 per cent in the choledochodochostomy group. This indicated that the latter anastomosis produced less obstruction ($p. < 0.025$).

	CHOLECYST-DUODENOSTOMY	CHOLEDOCHO-DOCHOSTOMY
ALLOGRAFT	7 (1a)	5 (1b)
AUTOGRAFT	7 (2a) ($p < 0.025$)	2 (2b)

AUTOPSY INCIDENCE OF CHOLESTASIS

Cholangitis was found in 65 per cent of the animals where the common bile duct was anastomosed to the duodenum (1a, 2a), but was virtually absent (5 per cent) when bile duct to bile duct anastomosis was employed (1b, 2b). Choledochodochostomy not only reduced the incidence of cholestasis, but also reduced the incidence of cholangitis.

	CHOLECYST-DUODENOSTOMY	CHOLEDOCHO-DOCHOSTOMY
ALLOGRAFT	9 (1a)	1 (1b)
AUTOGRAFT	4 (2a)	0 (2b)
	13 ($p < 0.001$)	1

AUTOPSY INCIDENCE OF CHOLANGITIS

DISCUSSION

The importance of cholangitis and intrahepatic sepsis has been discussed previously (page 66). It is postulated from the results presented that preservation of the sphincter of Oddi results in a significant reduction in biliary infection in this animal. Whether this information can be directly extrapolated to the human situation is debatable.

Starzl discusses at length the frequency and severity of biliary infections in human transplantation (166), but it is difficult to assess from the literature which of the methods already employed (choledochoduodenostomy, choledochodochostomy, cholecystoduodenostomy or cholecystojejunostomy) would be the most suitable. The reconstitution of the bile duct as suggested above has two possible disadvantages in the human. Firstly, the presence of a T tube could act as a portal for infection and, secondly, ischaemic necrosis leading to sloughing of the bile duct can occur and has been reported (166).

It was shown earlier that if cholecystduodenostomy alone was performed (animals 51 - 55), infection is virtually absent (page 45). These findings are broadly similar to those of Brettschneider et al. (19) and McSween (116), who suggest that liver damage (by auto- or allotransplantation) is a prerequisite to hepatic infection. Not only this, but it appears that cholestasis is minimal or absent in the animal with a normal liver and a cholecystduodenostomy. There are scanty and inconclusive reports in the literature of experimental cholecystduodenostomy and its consequences (111). It would appear that the trauma of ischaemia and perfusion put the liver at a grave disadvantage, such that defects of biliary drainage and infective assaults, usually tolerated by the normal liver, are in this situation followed by cholestasis and cholangitis.

The rejection process has been shown to be accompanied by biochemical and histological evidence of cholestasis in the human (155, 156), in the dog (90, 160, 159, 168, 99) and in the baboon (119). Cholestasis was not a feature of the porcine series of Calne (32) or Terblanche (175); it was, however, reported in this animal by Mieny (106) and

Starzl (166) in whose series rejection was marked. From the evidence presented in this study, it is suggested that cholestasis occurs pari passu with the rejection process and is usually directly proportional to it.

The precise nature of the cholestatic lesion is unknown. Electron microscopy has led to various postulates - either hepatocyte damage and consequent collapse of the canaliculi leads to mechanical obstruction, or distortion of canalicular microvilli is responsible for the changes (126). Myburgh (119), on the other hand, supports the hypothesis of Shaffner and Popper (170), postulating that intrahepatic cholestasis is a primary hepatocellular alteration of micellar secretion of bile salts. Hepatocyte injury, he claims, produces a hypertrophic but hypoactive smooth endoplasmic reticulum and a specific disturbance in bile salt metabolism. This hypothesis receives support from more recent work by Schaffner (150), who has shown that cholestasis is characterised by decreased metabolism of substrates associated with cytochrome system.

TABLE 9 SURVIVAL TIMES AND INCIDENCE OF COMPLICATIONS

		PIG	SURVIVAL (days)	ULCERATION	CHOLESTASIS	REJECTION	COMPLICATION OF ULCERATION
GROUP 1 (ALLOGRAFTS)	1a Cholecystduodenostomy	1	51	+	+	+	Haemorrhage, anaemia
		2	5	(+)	-	+	-
		3	46	(+)	-	+	Haemorrhage, death
		4	6	-	+	+	-
		5	21	+	+	+	Anaemia
		6	7	+	+	+	Haemorrhage, death
		7	13	+	+	+	Haemorrhage, death
		8	28	+	+	-	Haemorrhage, death
		9	8	+	-	-	Haemorrhage, death
		10	7	+	+	+	Haemorrhage, death
	1b Choledochodochostomy	11	13	(+)	-	-	-
		12	33	+	-	-	Haemorrhage, death
		13	18	+	+	-	Haemorrhage, death
		14	15	+	+	+	Perforation, death
		15	13	+	+	+	Haemorrhage, death
		16	7	-	-	+	-
		17	23	-	+	+	-
		18	24	+	-	+	Perforation, death
		19	38	-	-	+	-
		20	15	+	+	+	Haemorrhage, death
GROUP 2 (AUTOGRAFTS)	2a Cholecystduodenostomy	21	17	+	+		-
		22	75	-	-		-
		23	6	-	-		-
		24	7	+	+		Haemorrhage, death
		25	6	+	+		Haemorrhage, death
		26	12	+	+		Haemorrhage, death
		27	35	+	+		-
		28	9	+	-		Haemorrhage, death
		29	27	+	+		-
		30	28	+	+		-
	2b Choledochodochostomy	31	28	-	-		-
		32	24	-	-		-
		33	17	-	-		-
		34	16	(+)	+		-
		35	21	-	-		-
		36	18	-	-		-
		37	18	-	-		-
		38	17	-	-		-
		39	22	(+)	+		-
		40	76	-	-		-

( ) = microscopic only

7. CORRELATIONS

3. GASTRIC ULCERS, CHOLESTASIS AND TRANSPLANTATION

Gastro-intestinal haemorrhage has been recorded after human liver transplantation (148) and ulceration has been reported after experimental transplantation both in the dog (169) and the pig (175). This unusual phenomenon therefore allowed peptic ulceration to be examined in the new experimental model of the pig with a transplanted liver.

(i) The Incidence of Ulceration

The overall incidence of gastric ulceration in the transplant experiments (Groups 1a and 1b) on histological examination of the stomach was 80 per cent, with a mortality rate of 60 per cent from haemorrhage or perforation (Table 9). The type of biliary drainage made no statistical difference to the histological incidence (1a - 90 per cent, 1b - 70 per cent), or to the fatality rate (1a - 60 per cent, 1b - 60 per cent). The autograft control group, where cholecystduodenostomy had been employed (Group 2a) had a similar incidence of histological ulceration (80 per cent) and fatal ulceration (40 per cent). However, in the autograft control group with choledochodocostomy as biliary drainage (Group 2b), there was no macroscopic evidence of ulceration and the incidence of histological ulceration was reduced to 20 per cent without complications from these ulcers.

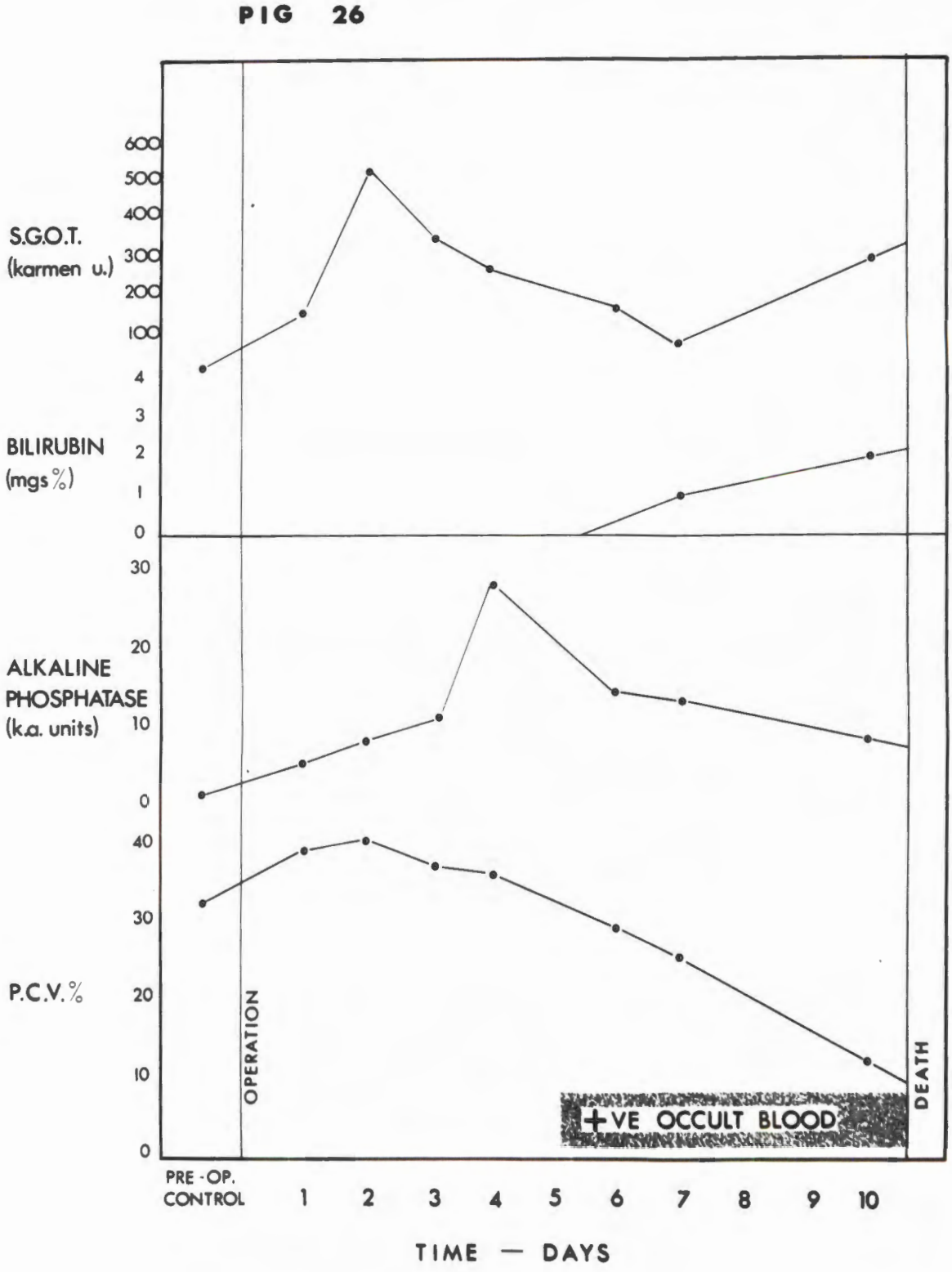


FIGURE 20: Profile of fatal ulceration (Animal 26).

(ii) Natural History of Ulceration and Complications

No gross incidence of ulceration was noted in animals kept for 3 weeks awaiting operation. Postoperatively the affected animals usually developed ulceration which was most commonly acute and fatal. Towards the end of the first postoperative week the animals became pale and lethargic and the stool occult blood positive. At this time the haematocrit and haemoglobin fell progressively (Fig. 20) and death usually occurred during the second and third postoperative weeks from massive gastric haemorrhage. Two animals died from frank intraperitoneal perforation of gastric ulcers. A less common picture (2 animals) was that of moderate gastric haemorrhage with a hypochromic microcytic anaemia.

FIGURE 21: Normal porcine stomach opened along greater curvature.

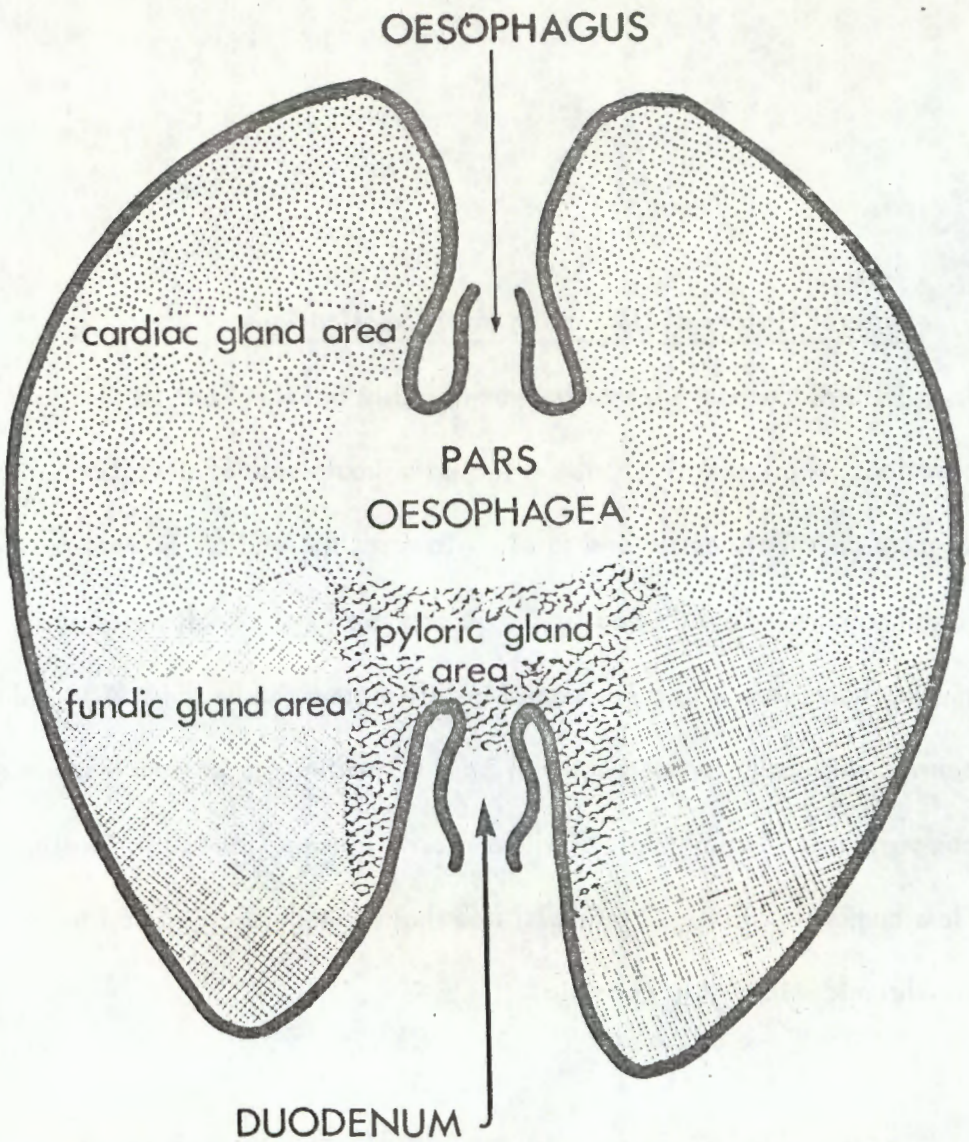


FIGURE 22: Gastric ulcer at pars oesophagea.

(Both figures by permission of the Editor, The Journal of Surgical Research)

(iii) Pathology of the Ulcers

All ulcers occurred at the pars oesophagea of the stomach, where a tongue of squamous epithelium forms part of the cardiac portion of the organ (Figs. 21, 22 and 23). At autopsy the organs were pale from exsanguination and the bowel usually contained up to a litre of altered blood. The ulcers were round or square in shape, usually about 3 cms. in diameter, but, in some animals, extending up to 7 cms. All ulcers were confined to the squamous pars oesophagea and no ulcer extended on to the glandular fundus or body of the stomach, nor into the oesophagus. The floor of the ulcer was usually grey or bile stained and contained necrotic debris. Frequently erosions or the open mouth of a vessel were observed at the base of the ulcer. Five ulcers were not apparent macroscopically.

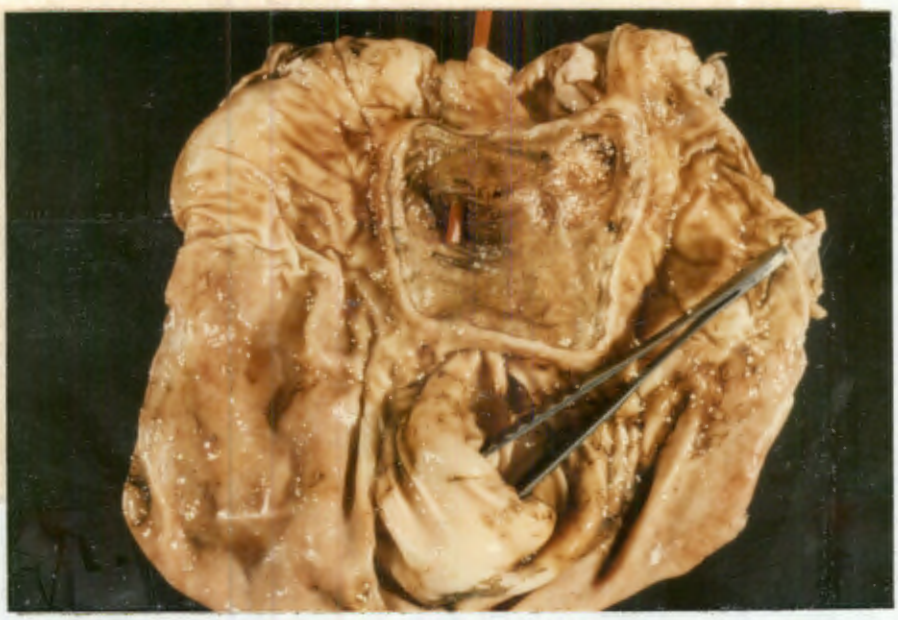


FIGURE 23: Gastric ulcer at the pars oesophagea.
(Red probe through oesophago-gastric junction, forcep through the pyloris).

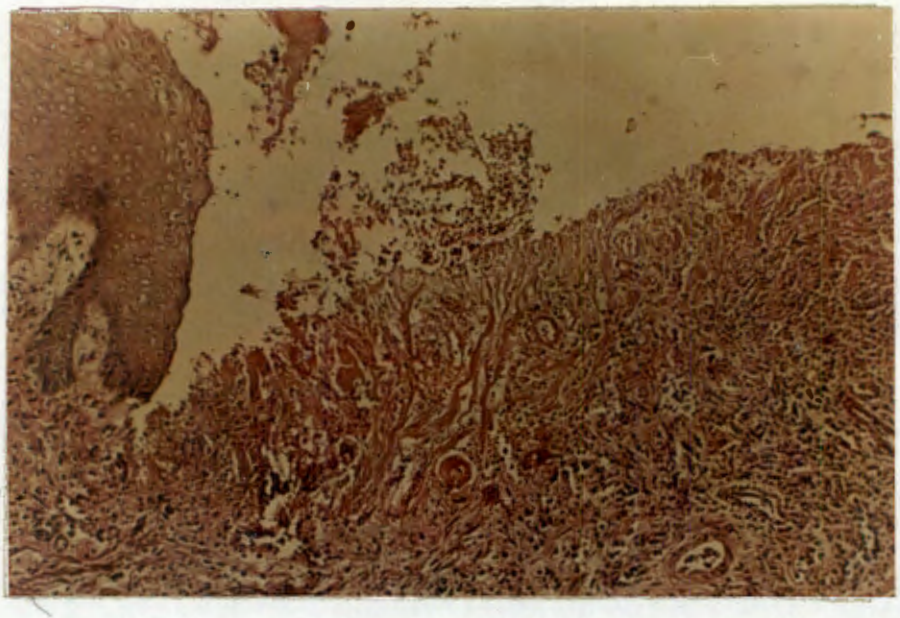


FIGURE 24: Edge of a large ulcer showing squamous epithelium and base of ulcer composed of newly formed fibrous tissue. There is a superficial zone of necrotic tissue and fibrin. Below this there is newly formed fibrous tissue in which smooth muscle bundles persist. (Haematoxylin and eosin x 120).

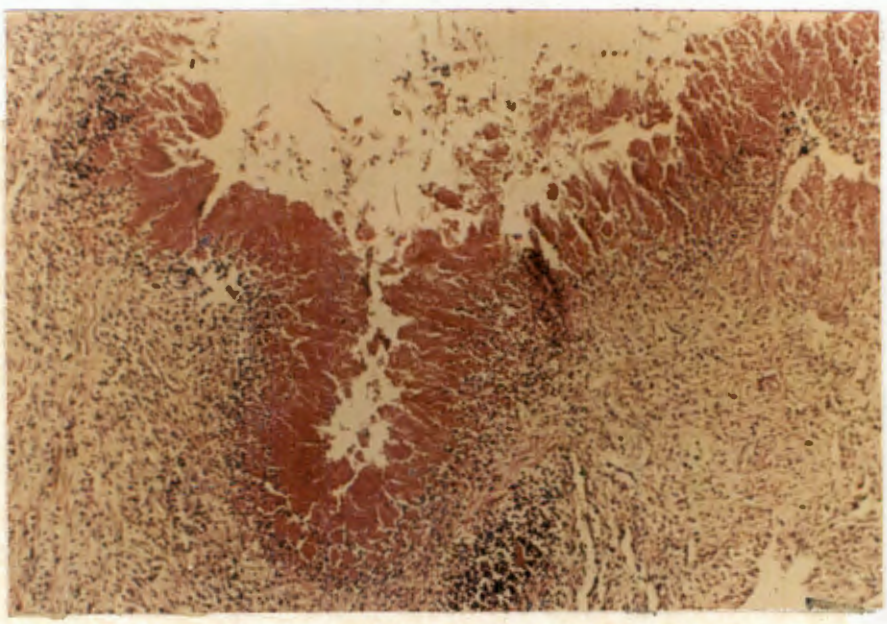


FIGURE 25: Small erosion of stomach. The adjacent squamous epithelium shows evidence of commencing regeneration. (Haematoxylin and eosin x 75).

(Both photomicrographs by permission of the Editor, The Journal of Surgical Research)

Microscopically the proximal edge of the large ulcers consisted of squamous epithelium (Fig. 24) and the distal edge of glandular stomach mucosa. The small ulcers, often detected microscopically only, were always placed in a region clothed entirely by stratified squamous epithelium (Fig. 25).

The majority of the ulcers were large and showed the classical features of subacute peptic ulceration (Figs. 24 and 25). Superficially, the base consisted of a narrow zone of eosinophilic fibrin, beneath which lay a zone of necrotic tissue showing the outlines of dead cells. This merged with, and was replaced by, a broad zone of newly-formed and vascularised young granulation tissue. Smooth muscle of stomach wall persisted externally to this tissue, and within it, to varying degrees. In only 2 cases was muscle entirely replaced by granulation tissue, as in a chronic peptic ulcer. Occasional small arteries, showing necrosis and partial or complete occlusion by organising thrombus, were noted within this tissue. Chronic inflammatory cells, including lymphocytes, plasma cells and macrophages, were present in the granulation tissue in small numbers. Two cases showed,within this tissue, acute suppurative foci and obvious septic thrombus formation in adjacent vessels.

The small erosions showed focal loss of squamous epithelium, with minimal underlying granulation tissue formation and inflammatory cell infiltration, confined to the submucosa. Usually the epithelium at the edge of these lesions showed signs of active regeneration. Indeed, foci of completely regenerated squamous epithelium, probably representing recently-healed erosions, were also detected not infrequently, indicating that the

ulceration was a recurrent process in which some ulcers enlarged and others healed soon after being initiated.

(iv) The Association of Cholestasis and Gastric Ulceration

Histological evidence of rejection, usually mild, was found in all allografts which developed gastric ulceration. In these animals cholestasis was a prominent associated feature, both biochemically, where the alkaline phosphatase and bilirubin were elevated, and histologically, where bile plugs and bile lakes were seen. In the autograft control Group 2a, where 80 per cent of animals developed ulceration, 70 per cent had evidence of cholestasis (Table 9). In the autograft control Group 2b, however, ulceration was evident in 2 animals on histological examination only. In this group only these 2 animals showed biochemical or histological evidence of cholestasis.

DISCUSSION

In man peptic ulceration is a well documented complication of portacaval shunt operations (70), as is gastro-intestinal haemorrhage after liver transplantation (148). Experimentally gastric hypersecretion and ulceration have been recorded in dogs after shunt procedures (28) and after liver transplantation. Terblanche et al. (175) noted that ulceration occurred at the oesophago-gastric junction after liver transplantation in the pig, a site where spontaneous ulceration has been reported in veterinary literature (92). Lesser curve ulceration, however, has been found in the pig, after the considerable gastric stasis produced by vagotomy without a drainage procedure, by Dragstedt and his associates (49). They found no ulcers at the pars oesophagea.

The abbatoir incidence of porcine gastric ulceration varies. Curtin et al. (45) reported an incidence of 19.64 per cent and O'Brien (121) reported 25.8 per cent. The fatal complication rate has been estimated at 4.59 per cent. Various factors have been implication in the pathogenesis of porcine gastric ulcers, and Kowalczyk (92) reviews dietary protein quality, hepatosis dietetica, copper poisoning, vitamin E lack, environment and high energy finely ground ration as possible aetiological factors. None of these factors appeared to be primarily operative in this series.

In this study, ulceration was found in 80 per cent of all animals undergoing liver transplantation, with a 60 per cent fatality rate. It also occurred in an autograft control group (2a) with the same natural history and incidence. This suggests that ulceration is not a complication specifically related to immunological rejection.

The onset of ulceration was rapid, sometimes as early as 5 days after transplantation, and usually within 14 days of the operation; the nature of the ulceration was vicious, with a high complication and fatality rate. In this series, both the onset and the incidence appeared to be associated with the onset and incidence of biochemical and histological cholestasis.

Partial or complete extrahepatic biliary obstruction appears to predispose to ulceration in the experimental animal. Silen et al. (139) demonstrated that gastric hypersecretion occurred in dogs after liver damage caused by degrees of obstruction to the common bile duct. Gastric ulceration and haemorrhage was produced in a series of pigs, by ligation of the bile ducts, by Bicknell et al. (7).

In the present series, partial biliary obstruction and cholestasis was assumed to be produced by cholecystduodenostomy in an autograft control group (2a) with subsequent ulceration. The cholestasis seen in the allograft group with cholecystduodenostomy (1a) could have been produced by the rejection process or by the partially obstructive biliary anastomosis. The cholestasis found in the allograft group with the choledochodochostomy (1b), however, must have been produced by the rejection process per se, for the control group of autografts (2b), with a similar biliary drainage system, showed minimal cholestasis. The association of hepatic rejection and cholestasis is well documented. It would appear that, in addition to extrahepatic biliary obstruction producing gastric ulceration, rejection induced intrahepatic cholestasis is associated with gastric ulceration.

Whatever the mechanism, cholestasis, whether of extra- or intrahepatic origin, is associated with some biochemical lesion in the liver resulting in gastric ulceration in this experimental model. The fact that the ulceration occurs at the non-glandular pars oesophagea would suggest increased acid-pepsin activity, as would the fact that it may be prevented by prophylactic vagotomy and drainage (29). It remains to be determined whether the biochemical lesion is one of inefficient degradation of histamine or gastrin and its analogues, or whether portasystemic shunting of these substances occurs.

CHAPTER V

SUMMARY AND CONCLUSIONS OF THE THESIS

The four problems posed in Chapter 11 at the outset of this study have been discussed variously in the appropriate sections. The conclusions reached will now be summarised.

(i) The natural history of the unmodified pig liver allograft

During the first 4 postoperative days, the operative trauma of liver transplantation consequent on manipulation, ischaemia, perfusion and hypothermia, resulted in a biochemical disturbance, notably release of S.G.O.T., which reached a peak at 24 hours and returned to near normal levels by the fourth day. In contrast, the alkaline phosphatase and albumin levels usually fell. The white cell and platelet counts were depressed over this period. Histological changes were minimal.

Fifteen per cent of animals underwent fatal rejection between 5 and 7 days. The stigmata of this process were similar to those described in other unimmunosuppressed species, namely, a progressive elevation of S.G.O.T., alkaline phosphatase and the appearance of jaundice. This was accompanied by the destructive histological picture of an immunological assault. There was a concomitant leucocytosis. This picture confirms the observations of other workers (175, 77, 166, 25, 106) that fatal rejection does occur in the pig and contradicts the contrary postulate of Calne (39).

The remaining animals underwent a milder form of rejection, usually in the second week, with moderate round cell infiltration and mild elevations of S.G.O.T., alkaline phosphatase and bilirubin at this time. These findings are similar to reports in the literature in the pig, without immunosuppression, and other species on immunotherapy. Serial examination of in vivo biopsy specimens showed that in many animals the round cell invasion was evanescent, usually maximal in the second week, and thereafter tending to subside in intensity. The biochemical profile tended to run a parallel fluctuating course.

Liver dysfunction was apparent for periods up to a month, as evidenced by hypoalbuminaemia and failure to gain weight. The similarity of this finding in the control groups suggested that the mechanism could be the non specific one of the trauma of ischaemia, perfusion and hypothermia, rather than a specific immunological process.

The overall mean survival time in this series was 21 days which, when compared with Starzl's survival time of 7.1 days in the unimmunosuppressed dog (160), would provide further evidence that the pig may undergo low grade rejection on occasion, resulting in extended survival.

It was concluded that the natural history of the unmodified pig liver allograft could manifest itself in two principal ways: firstly, a less common fatal rejection pattern, entirely comparable to that usually found in other animals, and, secondly, a more common form of mild rejection which is rare in other species. The immunological reason for this difference lay beyond this study and its aetiology remains speculative.

The fact that the longest survivor lived for 51 days and that the mean survival time for the whole group was 21 days, indicated that the ultimate outcome after liver transplantation was uniformly fatal. Although sporadic long term survivors have been reported in pigs (175, 31, 166), dogs (141, 144, 149, 167, 109) and humans (144, 158, 161, 37), the percentage yield, compared to the initial numbers is disconcertingly small. In all species and in all series, survival of over a year is highly exceptional. Moreover, the sinister implications of late cirrhosis and the extraordinary susceptibility of the animal to infection even without immunosuppression further mitigates long term survival.

It would appear at the present time that liver transplantation results in high morbidity and mortality and has an eventual fatal outcome. This picture would have to be carefully weighed against the known behaviour of human hepatic disease when this drastic therapy is considered in man. The need for further progress in this field is apparent.

(ii) Abnormalities specific to the rejection process

It is clearly essential that rejection should be able to be diagnosed rapidly and conclusively if the appropriate measures are to be taken to suppress it. For this reason specific biochemical and haematological patterns were sought by correlating simultaneous histological, haematological and biochemical observations. In addition, a series of control experiments were undertaken under a similar protocol so that, by deduction, the sequelae of the trauma of ischaemia, hypothermia, manipulation and

perfusion could be subtracted from the allograft parameters, thus leaving rejection-specific changes for appraisal.

In the bulk of the material examined, the histological changes of rejection were almost invariably accompanied by elevations of alkaline phosphatase, bilirubin and S.G.O.T. There was also a leucocytosis. This concurs with experience in all other species and appears to be the usual biochemical manifestation of hepatic rejection. Further evidence for the biochemical manifestation of rejection came from analysis of the mean values at the end of the first and second weeks - these biochemical modalities were found to be higher in the allograft groups, suggesting rejection specific changes. Moreover, the higher elevation of these biochemical modalities was usually accompanied by histological evidence of cholestasis. It is felt, therefore, that cholestasis, particularly in biochemical terms, accompanies the rejection process per se.

A proportion of animals dying from various causes were found to have disturbed biochemistry terminally. This was found to be associated with hepatic zonal necrosis and is thought to be a consequence of agonal under-perfusion. The misinterpretation of this event as terminal rejection has been pointed out.

While the association of a cholestatic biochemical profile and rejection was confirmed, it was disappointing to find that this association was non specific. In this study liver cell necrosis and bile duct obstruction were found to produce biochemical patterns similar to rejection. These mimics could also confusingly produce fluctuating profiles,

which could falaciously suggest rejection. This was thought to be particularly significant in that there are many additional causes of postoperative liver dysfunction after human liver transplantation. These include initial ischaemic injury to the graft in the donor, infarction from vascular insufficiency, extrahepatic biliary obstruction, cytomegalic inclusion disease, serum hepatitis and Imuran hepatitis.

It was concluded that elevations of S.G.O.T., alkaline phosphatase and bilirubin were suggestive of rejection, but liver biopsy provided the only finite answer. An early specific quantitative biochemical test for rejection is clearly desirable in liver transplantation.

(iii) The most satisfactory form of biliary drainage

Two forms of biliary drainage were tested in this animal - choledochodochostomy and cholecystduodenostomy. The outcome was assessed in terms of biochemical and histological evidence of cholestasis, and the incidence of cholangitis.

When the biochemistry was analysed at the end of the first and second weeks in the autograft controls (where the cholestatic manifestations of rejection could not confuse the picture), the cholecystduodenostomy group had significantly higher parameters than the choledochodochostomy group, suggesting that the former anastomosis produced cholestasis. The histological incidence of cholestasis at autopsy confirmed this observation ($p < 0.025$). This suggested that reconstitution of the bile duct produced less mechanical ductal obstruction than anastomosis of the gall bladder to the duodenum.

In addition, the reduction of cholangitis by choledochodochostomy was statistically significant ($p < 0.001$), suggesting that preservation of the sphincter of Oddi reduced the incidence of biliary infection.

It was concluded, therefore, that choledochodochostomy was the most satisfactory form of biliary drainage in the pig. Whether this information can be directly extrapolated to the human situation is debatable, for, not only would a T-tube (with its possibility of infection) probably have to be used, but the blood supply to the bile duct in the human appears less abundant and sloughing has been reported (166). Choledochoduodenostomy, choledochodochostomy, cholecystduodenostomy and cholecystjejunostomy have variously been employed in human transplantation. The superiority of any particular method is not apparent from the human literature.

(iv) The complications : biliary stasis, cholangitis, infection and peptic ulceration

The complications of biliary stasis and cholangitis were found to be common and have been shown to be reduced by preservation of bile duct continuity after transplantation in the pig.

Thirty-five per cent of animals died from infection, the most common type being a bilateral pneumonia occurring between 17 and 35 days. The organisms were multiple combinations of gram negative bacilli and these have been shown to be normal commensals of the gastro-intestinal tract. The frequency of pulmonary infection and gram negative bacillus complications in the literature is noted. It is felt that the portal

vein provides a significant route of systemic entry for organisms when the liver is put at a disadvantage by the trauma of ischaemia and perfusion. This handicap conferred on the liver by ischaemia and perfusion, and the liver's strategic position in the prevention of systemic infection, was emphasised by two observations: Firstly, the infection seen in Starzl's series had been partly attributed to the immunosuppression used; in this present study, however, no immunosuppression was used, yet infection was nevertheless prominent. Secondly, infection occurred in a significantly high proportion of the control animals where rejection could play no part.

Gastric ulceration was found in 80 per cent of the allografts, with a 60 per cent fatality rate. The ulceration occurred exclusively at the oesophagogastric junction 5 to 14 days after transplantation and was frequently complicated by fatal haemorrhage or perforation. The biochemical, haematological and morphological changes associated with it were recorded. The fact that ulceration was found to be of equal incidence in a group of autograft controls, with cholecystduodenostomy as biliary drainage, suggested that this complication was not specifically related to immunological rejection. An association was noted between the incidence of ulceration and that of biochemical and histological cholestasis, whether the cholestasis was produced by extrahepatic biliary obstruction or by an intrahepatic rejection lesion. The aetiology of the ulcers remains speculative. It should be determined whether the biochemical lesion is one of inefficient degradation of histamine or gastrin and its analogues, whether portasystemic shunting of these substances occurs, or whether another mechanism is responsible.

DETAILED RESULTS

Appendix (a)

UNITS AND ABBREVIATIONS USED

BILIRUBIN:	milligrams/100 ml.
ALK.PHOS:	Alkaline Phosphatase, Shinowara-Jones-Reinhart units
SGOT:	Serum Glutamic Oxaloacetic Transaminase, karmen units
T.PROT:	Total Protein, grams per cent
ALB:	Albumin, grams per cent
GLOB:	Globulin, grams per cent
PCV:	Packed Cell Volume, per cent
WBC:	White Cell Count, x 1000 $mm.^3$
PLAT:	Platelets, x 1000 $mm.^3$
HISTOLOGY:	Menghini needle biopsy (salient features)
* :	Day biopsy taken
P.M. :	Post mortem morbid anatomy
Histo:	Histology (salient features)
- :	Specimen missing
+ :	Histological feature present
++ :	Histological feature marked
- :	Histological feature absent

Pig. No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
1.	0	0	0	4.1	38	5.31	2.31	3.00	29	9.8	-	
	1	1.3	0	17.2	1500	5.30	2.01	3.49	33	21	-	
	2	2.3	1.8	20.2	1300	4.80	2.15	2.65	31	11.2	-	
	3	0.5	0	16.2	620	4.86	2.01	2.85	30	10.3	-	
	4	1.8	1.1	13.1	230	5.00	2.00	3.00	29	10.4	-	
	5	1.0	0.5	5.0	190	5.25	1.95	3.30	29	10.5	-	*Cholestasis,
	6	1.0	0.5	4.8	98	5.22	1.90	3.32	30	10.4	-	focal necrosis
	7	2.0	1.0	9.5	85	5.00	1.59	3.40	32	15.2	-	
	8	2.2	1.7	8.0	94	5.06	1.61	3.45	32	22	-	
	9	7.2	5.4	9.0	130	5.64	1.64	4.00	28	38	-	
	13	2.4	2.1	5.8	120	4.77	1.84	3.00	22	34	-	
	14	2.0	1.2	-	210	4.90	1.70	3.20	21	37.6	-	
	15	2.3	1.9	-	180	4.77	1.72	3.05	20	34.3	-	*As above, +
	20	1.6	1.1	5.8	110	6.22	2.21	4.01	20	21	-	monos, plasma cells
	28	8.3	6.4	17.1	210	5.66	2.20	3.46	22	32.9	-	
	34	2.8	1.6	15	95	6.60	2.21	4.20	24	31.3	-	*Cholestasis,
	37	9.5	9.7	6.1	290	5.6	2.20	3.30	-	-	-	scanty monos
	48	6.2	6.1	8.5	60	6.30	2.10	4.10	22	38.6	-	
	51	P.M. Emaciated, jaundiced, all wounds infected, gastric ulceration. Histo: Rejection+ (less than before), cholestasis+, no cholangitis.										
2.	0	0	0	6.0	35	4.66	1.59	3.07	27	24	198	
	1	1.2	1.0	11.7	1240	4.23	1.62	2.61	38	24.9	102	
	4	1.5	1.4	9.5	190	4.09	1.5	2.59	35	52	110	
	5	6.8	6.3	6	46	4.50	1.6	3.90	45	51.5	94	
	5	P.M. Macroscopically normal. Histo: Rejection++ (diffuse round cell infiltration, liver cell necrosis, parenchymal disruption), cholangitis+, no cholestasis, microscopic ulceration.										

Pig No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
3.	0	0	0	6.2	23	6.15	2.14	4.01	34	15	-	
	1	0	0	8.2	740	4.66	1.56	3.1	48	22	-	
	2	0	0	7.7	290	4.97	1.15	3.82	48	18	-	
	3	0	0	7.7	170	6.66	1.29	3.37	47	11.7	-	
	4	0	0	5.6	150	6.14	1.95	4.19	-	11	-	
	5	3.2	1.7	9.2	120	-	-	-	-	-	-	
	6	3.1	1.7	9.3	100	6.07	1.81	4.2	50	24.8	-	
	7	0	0	7.2	80	6.6	1.88	4.7	48	23.9	-	
	8	0	0	7.2	50	6.6	1.9	4.7	48	23.9	-	*Plasma cells, monos
	13	0	0	-	100	6.4	1.9	4.5	-	-	-	
	14	0	0	13.3	102	-	-	-	48	26.7	-	
	21	0	0	2.9	50	-	-	-	31	34.3	-	
	24	0	0	12.2	85	5.35	1.3	4.05	25	39.3	-	
	35	0.8	0.5	15	100	-	-	-	27	65.5	-	*More intense round cell infiltration, necrosis
	45	2.2	1.8	18	810	5.17	1.13	4.04	26	66	-	
	46	P.M. Emaciated animal, enlarged nutmeg liver, blood in bowel, no ulcer evident. Histo: Centrilobular necrosis, rejection+ (including many plasma cells), regeneration, damage, no cholestasis, cholangitis+, vascular changes, microscopic ulceration.										
4.	0	0	0	2.8	35	6.07	2.04	4.02	35	5.8	469	
	1	0	0	4.5	320	5.55	1.55	4	36	6.7	472	
	2	0	0	3.1	140	4.9	1.41	3.5	32	18.3	468	
	3	1.0	0	4.0	98	4.05	1.52	3.52	30	20.1	453	
	4	0.7	0.6	4.0	275	5.49	1.79	3.6	28	25.8	443	*Plasma cells, monos, necrosis
	6	1.4	1.0	6	460	5.93	1.93	4	50	75.2	291	
	6	P.M. Normal. Histo: Rejection++ (dense monocyte infiltration, loss of liver cells, mitoses), cholestasis+, cholangitis+, no ulceration.										

Pig No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
5.	0	0	0	4.0	32	6.44	2.17	4.27	38	17	144	
	1	0	0	5.1	245	3.91	1.23	2.68	43	21.2	182	
	7	1.2	0.8	6.8	105	-	-	-	33	21	426	*Cholestasis,
	15	2.1	1.9	2.8	145	-	-	-	20	46	348	scanty round cells
	21	P.M. Bilateral pneumonia, hard knobbly liver, pus in bile ducts, gastric ulceration. Histo: Rejection+, cholestasis+, cholangitis+.										
6.	0	0	0	6.1	20	5.75	2.07	3.67	27	17.4	502	
	1	0	0	9.6	290	4.68	1.79	2.89	26	15.8	409	
	3	0	0	5.0	35	5.21	1.54	3.67	29	16.3	450	
	4	0	0	4.2	120	5.46	1.71	3.75	31	21.1	242	*Essentially
	7	5.2	4.7	6.7	170	-	-	-	12	25.5	440	normal
	7	P.M. Gastric ulcer, bowel filled with blood. Histo: Rejection+ (plasma cells, eosinophils, necrosis, mitosis), cholangitis+, cholestasis+.										
7.	0	0	0	4.1	40	5.46	1.84	3.62	40	64	-	
	3	0	0	2.8	110	4.57	1.71	3.86	44	12	-	
	7	0	0	0.9	70	4.57	1.26	3.31	32	32	-	*Monos, mitosis
	10	0	0	3.6	30	4.61	1.28	3.33	16	47	315	*Monos, mitosis (More than above)
	13	P.M. Gastric ulcer, bowel filled with blood. Histo: Centrilobular necrosis, rejection+, cholangitis+, cholestasis+.										
8.	0	0	0	1.6	30	5.8	2.06	3.74	28	12	280	
	1	0	0	11.1	-	3.91	1.22	2.69	26	27.8	260	
	2	0.9	0	7.3	1200	3.84	1.22	2.62	25	16.7	280	
	3	0	0	5.3	110	4.36	1.01	3.35	27	12.5	251	
	6	1.8	1.2	3.5	180	5.06	1.15	3.91	26	12.9	650	
	8	2.4	1.5	2.1	160	5.9	1.15	4.75	26	23.9	440	*Monos, plasma cells, cholestasis

Pig No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
8 (cont.)	16	0	0	2.1	20	-	-	-	32	28.8	550	*Mild monos
	23	0	0	2.4	68	7.09	4.5	2.59	27	32.8	150	*Mild monos,
	27	1.3	1.1	2.2	45	6.8	4.78	2.02	31	32	122	bile plugs
	28	P.M.	Emaciated, gastric ulcer, blood in bowel, nutmeg liver.									
		Histo:	Cholestasis+, cholangitis+, necrosis, rejection-.									
9.	0	0	0	4.0	75	-	-	-	25	12	200	
	1	0	0	8.9	660	4.81	1.40	3.41	29	25.3	211	
	4	1.4	0.8	4.5	100	5.23	1.40	3.83	27	23.9	400	
	6	3.4	2.7	6.8	170	5.97	1.60	4.37	30	38	236	*No rejection
	8	P.M.	Gastric ulcer, bowel filled with blood.									
		Histo:	Centrizonal necrosis, cholangitis+, no cholestasis or rejection.									
10.	0	0	0	4.3	25	6.21	2.04	4.29	29	17.8	530	
	2	0	0	3.3	145	5.51	1.36	4.29	23	17.1	300	
	3	0	0	2.1	50	5.62	1.36	4.29	25	15.4	357	
	4	0	0	2.1	140	5.86	1.61	4.25	29	11.4	281	
	5	0	0	3.0	90	-	-	-	25	19.6	371	
	7	P.M.	Gastric ulcer, bowel filled with blood.									
		Histo:	Cholestasis+, cholangitis+, rejection+.									
11.	2	1.3	1.2	9.8	520	4.72	1.53	3.19	32	24	133	
	6	3.2	2.7	7.4	320	4.50	0.75	3.75	36	26.8	381	*Monos, mitoses
	13	P.M.	Wound infection, gaseous liquifaction of liver.									
		Histo:	Polymorphs, haemorrhages, necrosis, monocytes, no cholestasis, cholangitis or rejection.									
			Superficial gastric ulceration.									

Pig No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
12.	7	2.9	2.4	5.2	120	7.23	1.94	5.29	32	12.2	302	*Mitoses only
	15	0	0	9.0	200	7.39	-	-	35	24.6	242	*Patchy monos
	30	0	0	16.8	40	6.53	1.11	4.42	20	14.8	490	*Few monos
	33	P.M. Gastric ulcer, blood in bowel. Histo: Few plasma cells, no cholestasis, cholangitis or rejection.										
13.	7	3.0	2.5	10	230	5.43	1.71	4.72	36	34.6	387	*No monos
	17	2.7	2.2	3.2	90	6.50	1.43	5.13	38	23.2	257	*Few monos, zonal necrosis cholestasis
	18	P.M. Gastric ulcer, blood in bowel. Histo: Cholestasis+, no cholangitis or rejection.										
14.	7	1.3	1.1	6.0	75	6.21	1.29	4.92	33	12	686	*Monos
	14	0	0	3.2	70	5.62	1.32	4.30	30	13	164	*Heavy monos, plasma cells, eosinophils, cholestasis
	15	P.M. Perforated gastric ulcer. Histo: Cholestasis+, no cholangitis, rejection+ (monos and eosinophils).										
15.	11	0	0	8.8	115	6.07	1.36	4.71	33	24.8	338	*Mitoses, monos
	13	P.M. Gastric ulcer, bowel filled with blood. Histo: Cholestasis+, cholangitis-, rejection+ (monos, vascular changes).										
16.	0	0	0	4.8	35	4.70	1.62	3.08	27	11.4	240	
	6	4.0	3.2	13.2	220	5.46	1.20	4.26	29	28.3	302	
	7	P.M. Normal. Histo: Rejection++ (dense monocyte infiltration), no cholestasis or cholangitis.										
17.	6	1.6	1.4	10.8	120	4.47	1.15	3.32	34	12	320	*Mitoses, monos
	12	0.8	0.7	5	45	5.51	1.30	4.21	28	41.5	470	*Rejection
	21	0.9	0.8	7	180	-	-	-	31	18.1	275	*Monos, cholestasis
	23	P.M. Normal. Histo: Cholestasis+, far less rejection, no cholangitis. No cause for death found.										

Pig No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
18.	7	0.9	0.5	6.2	45	4.92	1.59	3.33	28	-	-	*Monos
	14	0	0	8.4	45	5.38	1.23	4.15	24	-	-	
	17	0	0	12.9	92	-	-	-	18	-	-	*Mild monos
	24	P.M. Perforated gastric ulcer.										
		Histo: Rejection+, no cholangitis or cholestasis.										
19.	8	0.6	0.4	8.4	50	5.55	1.85	3.70	37	28	432	*Some rejection
	19	0	0	6.2	55	6.67	1.82	4.85	34	18.2	642	*Rejection,
	27	0	0	5.2	55	6.00	1.52	3.48	30	16.4	777	(less obvious)
	36	0	0	6.0	35	6.88	1.98	4.90	34	11.6	223	
	38	P.M. Large, obstructed ventral hernia.										
		Histo: Minimal rejection, no cholestasis, cholangitis or gastric ulceration.										
20.	7	2.9	2.8	7.6	70	4.81	1.70	3.11	28	16.2	248	*Monos
	15	P.M. Gastric ulcer, blood in bowel.										
		Histo: Less rejection, cholestasis+, cholangitis+.										
21.	0	0	0	11.6	50	7.4	2.8	4.6	30	17.6	243	
	1	1.8	2.0	10.7	290	5.1	1.5	3.6	34	-	-	
	3	2.2	2.3	3.6	185	6.65	1.86	4.79	35	37.3	373	Focal necrosis,
	4	1.2	1.3	3.8	120	5.74	1.71	4.03	36	35	350	*cholestasis.
	7	0	0	3.7	70	5.73	1.03	5.7	37	32	257	*Ditto, cholangitis.
	11	2.0	2.4	3.1	55	5.73	1.39	4.34	30	48.4	334	*Cholestasis,
	17	0	0	3.0	38	5.75	1.40	4.35	32	40	264	cholangitis
	-	0	0	4.0	42	6.80	2.32	4.48	30	28	284	
	17	P.M. Pneumonia, gastric ulcer, generalised abscesses.										
		Histo: Cholestasis+, cholangitis+.										

Pig No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
22.	7	0	0	1.9	130	6.24	1.67	4.57	34	17.1	300	
	13	0	0	2.8	90	-	-	-	34	12.8	320	*Normal
	25	0	0	4.5	45	6.73	1.57	5.16	-	-	-	*Normal
	45	0	0	4.2	75	7.50	1.76	5.74	34	21.2	224	*Normal
	75	P.M.	Normal.									
		Histo:	Interlobular fibrosis, no cholestasis, cholangitis or ulceration.									
23.	0	0	0	2.9	-	-	-	-	38	12.1	154	
	1	0	0	9.6	960	6.2	1.39	4.8	35	25	150	
	2	0	0	6.1	170	5.1	1.24	3.86	38	30	151	
	3	0	0	5.8	75	5.13	1.31	4.22	39	37	178	
	6	P.M.	Pulmonary embolus.									
		Histo:	No cholestasis, cholangitis or ulceration.									
24.	0	0	0	3.8	71	6.3	-	-	34	9.8	-	
	1	0	0	9.4	330	4.2	-	-	36	10.2	-	
	2	0	0	-	245	5.4	-	-	32	11.4	-	
	7	P.M.	Gastric ulcer, bowel filled with blood.									
		Histo:	Cholestasis+, no cholangitis.									
25.	0	0	0	5.1	40	5.78	2.82	2.86	33	16.2	369	
	1	2.5	2.1	14	1455	5.43	2.36	3.07	40	14.7	302	
	2	2.6	0.9	13.7	1940	5.07	1.75	3.22	43	24.8	215	
	3	2.2	1.7	7.4	318	5.40	1.90	3.5	40	11.3	291	
	4	1.7	1.3	6.2	70	6.19	2.17	4.02	42	24.4	298	*Cholestasis,
	5	2.7	1.8	3.5	75	-	-	-	20	-	-	focal necrosis
	6	P.M.	Gastric ulcer, blood in bowel.									
		Histo:	Centrizonal necrosis, cholestasis+, no cholangitis.									

Pig No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
26.	0	0	0	1.5	30	6.14	2.39	3.75	33	17.5	457	
	1	0	0	5.7	145	5.57	2.28	3.29	40	25.5	217	
	2	0	0	8.5	455	5.36	1.60	3.76	45	37.6	225	
	3.	0	0	11.2	344	5.02	1.56	3.46	40	39.2	-	*Cholestasis,
	4	0	0	29.5	290	6.64	1.71	4.93	35	42.6	438	focal necrosis
	6	0	0	15.1	198	5.21	1.55	3.66	30	49.6	400	
	7	1.5	1.0	14.3	110	5.19	1.91	3.28	26	10	940	*Cholestasis,
	10	2.4	1.8	9.9	305	4.09	1.27	2.82	13	14	449	focal necrosis
	12	P.M. Gastric ulcer, blood in bowel, infected wound, obvious cholangitis.										
		Histo: Centrizonal necrosis, cholestasis+, cholangitis+.										
27.	0	0	0	2.7	50	6.0	2.32	3.68	30	30	328	
	1	0	0	6.2	410	4.3	1.29	3.01	42	30.4	327	
	2	0	0	4.2	250	4.87	1.48	3.4	-	-	-	
	4	0	0	5.0	85	5.44	1.54	3.9	35	14.4	290	
	5	0	0	3.6	70	6.42	1.80	4.62	34	20	465	
	8	0	0	1.8	40	5.08	1.54	3.44	25	36.7	-	*Normal
	15	0	0	1.2	35	4.73	1.17	3.6	25	27.6	703	*Normal
	23	0	0	3.8	30	5.49	1.19	4.2	-	-	-	*Normal
	30	0	0	5.2	-	-	-	-	29	34	376	
	35	P.M. Bilateral pneumonia, gastric ulceration.										
		Histo: Centrizonal necrosis, cholestasis+, cholangitis+.										
28.	0	0	0	2.9	38	6.38	1.56	4.82	30	25	327	
	1	0.4	0.2	16.3	2120	4.64	1.67	2.97	37	45.2	321	
	2	0	0	9.2	395	3.81	1.52	2.29	33	-	400	
	4	0	0	4.3	169	4.50	1.36	3.14	31	40.4	253	
	5	0	0	10.5	512	4.84	1.27	3.57	32	30	425	
	6	0	0	10.3	445	4.55	1.20	3.35	31	37.8	202	
	8	2.5	2.3	13	655	4.02	1.13	2.89	11	66.1	-	*Centrizonal necrosis
	9	P.M. Gastric ulcer, blood in bowel.										
		Histo: Centrizonal necrosis, no cholestasis or cholangitis.										

Pig No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
29.	0	0	0	5.6	25	5.6	2.7	2.9	30	21.4	224	
	4	0	0	2.4	35	5.01	2.79	2.22	38	36.8	-	
	11	1.2	1.1	1.1	50	5.67	1.67	4.0	48	35	260	*Cholestasis
	19	0	0	2.1	130	4.51	1.37	3.14	22	31	122	
	26	2.5	2.3	1.2	260	-	-	-	18	9.8	133	*Cholestasis, zonal necrosis
	27	P.M.	Atelectasis, pneumonia, wound infection, gastric ulceration.									
		Histo:	Centrizonal necrosis, cholestasis+, cholangitis+.									
30.	0	0	0	5	40	4.94	1.69	3.25	30	22.5	426	
	1	0	0	7	1720	2.79	0.74	2.05	36	27.2	284	
	28	P.M.	Wound infection, perihepatic abscesses, gastric ulceration.									
		Histo:	Cholestasis+, no cholangitis.									
31.	0	0	0	2.7	42	5.41	2.11	3.30	-	-	-	
	1	0	0	7.0	400	3.69	1.25	2.44	32	18.9	210	
	2	0	0	5.2	250	4.42	1.25	3.17	37	22.7	389	
	3	0	0	-	72	-	-	-	-	-	-	
	4	0	0	2.6	40	5.90	1.62	4.28	40	36	530	*Normal
	7	0	0	2.8	35	6.20	1.67	4.53	35	24	280	
	14	0	0	2.7	38	-	-	-	28	29	290	*Normal
	16	0	0	-	35	6.2	1.2	5.0	-	-	-	
	21	0	0	2.7	50	5.16	1.13	4.03	36	32	656	*Normal
	26	0	0	-	-	4.84	0.95	3.89	31	32	122	
	28	0	0	5.1	45	-	-	-	38	34.2	148	
	28	P.M.	Diffuse pneumonia with effusions.									
		Histo:	No cholestasis, cholangitis or ulceration.									
32.	1	0	0	2.4	45	4.45	1.42	3.03	31	20.9	331	
	3	0	0	1.4	20	5.51	1.71	4.80	30	21.7	201	
	8	0	0	1.3	10	6.03	1.63	4.40	31	23.8	276	*Normal

Pig No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
32 (cont.)	17	0	0	1.8	105	7.47	1.02	6.45	31	14.5	403	*Normal
	24	0	0	2.4	94	-	-	-	30	15.6	382	
	24	P.M.	Diffuse pneumonia, effusions, right subphrenic abscess.									
		Histo:	No cholestasis, cholangitis or ulceration.									
33.	0	0	0	4.1	10	5.57	1.83	3.74	37	23.7	329	
	2	0	0	4.1	115	5.42	1.77	3.65	-	22.2	242	
	10	0	0	1.6	68	-	-	-	14	18.3	387	*Normal
	17	P.M.	Pneumonia, wound infection.									
		Histo:	Zonal necrosis, no cholestasis, cholangitis or ulceration.									
34.	0	0	0	2.9	38	-	-	-	34	10.2	273	
	7	0	0	2.6	35	6.1	1.5	4.6	31	14.7	402	*Few plasma cells
	14	0	0	3.6	85	5.93	1.56	4.37	30	24	248	
	16	P.M.	Normal.									
		Histo:	Cholestasis+, no cholangitis, zonal necrosis, superficial gastric ulceration.									
35.	1	0	0	2.4	70	5.6	1.57	4.03	35	9	426	
	9	0	0	2.2	12	6.32	1.88	4.44	32	8.7	347	*Few plasma cells
	13	0	0	1.9	30	6.1	1.77	4.33	32	6.4	362	*Ditto
	21	P.M.	Bilateral pneumonia.									
		Histo:	Zonal necrosis, no cholestasis, cholangitis or ulceration.									
36.	1	0	0	3.5	220	-	-	-	-	16.4	372	
	2	0	0	4.2	110	-	-	-	28	15.2	353	
	4	0	0	2.6	90	5.9	1.47	4.43	26	18.2	392	
	7	0	0	1.9	45	5.59	1.36	4.23	22	22.4	302	*Normal
	15	0	0	1.5	32	6.99	2.34	4.65	17	25.4	152	*Normal
	18	P.M.	Normal.									
		Histo:	No cholestasis, cholangitis or ulceration.									

Pig No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
37.	0	0	0	2.9	21	6.63	2.13	4.5	27	13.8	265	
	1	0	0	7.8	2400	4.01	1.15	2.86	27	24	291	
	5	0	0	4.5	35	6.07	1.62	4.45	29	17.7	383	
	6	0	0	2.4	20	5.24	1.42	3.82	28	23	630	*Few lymphos.
	8	0	0	1.7	30	6.33	1.38	4.95	28	38	721	
	13	0	0	1.4	10	7.87	1.23	6.64	28	12.4	588	
	18	P.M.	Pneumonia, pleural effusions.									
		Histo:	No cholestasis, cholangitis or ulceration.									
38.	1	0	0	3.3	115	5.1	1.47	3.63	-	-	-	
	8	0	0	3.1	22	5.64	1.17	4.47	30	-	368	*Few polys
	14	0	0	2	25	6.79	1.16	5.63	-	-	-	*Normal
	17	P.M.	Bilateral pneumonia.									
		Histo:	No cholestasis, cholangitis or ulceration.									
39.	1	0	0	3.7	85	5.6	1.74	3.86	30	28.7	190	
	7	0	0	2.0	30	5.36	2.02	3.34	25	24.6	240	
	14	6.3	5.4	2.6	29	4.98	1.19	3.79	15	29.1	221	*Cholestasis
	21	4.2	3.6	6.4	190	5.81	0.89	4.92	36	41.7	294	*Cholestasis, zonal necrosis
	22	P.M.	Diffuse pneumonia.									
		Histo:	Centrilobular necrosis, cholestasis+, no cholangitis, superficial ulceration.									
40.	1	0	0	1.3	30	5.63	1.50	4.13	30	13.2	406	
	14	0	0	3.1	40	5.66	1.56	4.10	32	13.8	-	*Normal
	18	0	0	4.9	55	6.11	1.78	4.33	30	12.5	271	*Normal
	33	0	0	7.6	80	7.75	1.50	6.25	32	14.2	-	*Normal
	76	0	0	5	32	7.84	2.20	5.64	34	6.2	294	
	76	P.M.	(Sacrifice) Normal.									
		Histo:	Normal.									

Pig. No.	DAY	BILIRUBIN		ALK.PHOS	SGOT	T.PROT	ALB	GLOB	PCV	WBC	PLAT	HISTOLOGY
41.	7	0	0	4.9	42	6.92	2.8	4.12	32	20.9	436	
	14	0	0	3.1	37	6.75	2.91	3.84	38	12.4	155	
	98	0	0	6.2	36	7.02	3.22	3.80	35	11.2	165	
	98	P.M.	Normal.	(Sacrifice).								
		Histo:	Normal.									
42.	7	0	0	3.3	45	5.21	2.41	2.8	28	17.5	345	
	14	0	0	4.7	35	5.58	2.66	2.92	31	11.6	394	
	98	0	0	5.4	35	5.40	2.57	2.83	32	10.4	362	
	98	P.M.	Normal.	(Sacrifice).								
		Histo:	Normal.									
43.	7	0	0	5.3	64	6.32	2.92	3.4	-	-	-	
	14	0	0	7.0	38	5.95	3.25	2.7	25	12.9	398	
	98	0	0	3.9	37	6.75	3.38	2.37	38	19	139	
	98	P.M.	Normal.	(Sacrifice).								
		Histo:	Normal.									

Appendix (b)

FIRST REPORT OF ORGAN TRANSPLANTATION (46).

the drainage area from which the river is fed. The nitrates are a little higher than is usual in May, but the free and albuminoid ammonias compare very well with the results obtained by the New Orleans City Sewerage and Water Board. The silt varies very largely from month to month, hence no reliable conclusion can be drawn from any one analysis. This silt was saved and will be subjected to a plant food analysis at a later date.

In conclusion, let me say that this analysis has, to my mind, demonstrated the desirability of a very complete and detailed chemical study, month by month, of the Mississippi River and its tributaries, and I should have undertaken such a study personally had I not learned that it was already planned for by Mr. M. O. Leighton, in charge of the Division of Hydro-economics, U. S. Geological Survey.

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FUNCTIONS OF A TRANSPLANTED KIDNEY.

THE state of the circulation and of the secretion of a transplanted kidney has been observed on an animal operated on in this laboratory. A careful investigation of the literature has revealed no mention of a similar experiment having been performed hitherto.

The kidney of a small-sized dog was extirpated and transplanted into the neck. The renal artery was united to the carotid artery, the renal vein to the external jugular vein and the ureter to the œsophagus. Three days after the operation the neck and the abdomen were opened, in order to study the functions of the transplanted kidney and to compare them with the functions of the normal kidney. The transplanted kidney was found adherent to the muscles, and dissection was necessary to free it. In size it was larger than the normal kidney. Its hue was darker. To the touch the consistency of its tissue was normal, and the pulsations of its artery were as strong as the pulsations of the artery of the normal kidney.

Here is the summary of this observation: *the circulation in the transplanted kidney* was slightly greater than in the normal kidney, as detected by the touch, copiousness of hemorrhage from incision in cortex, and pulse-tracings.

The secretion of urine by the transplanted kidney was about five times more rapid than by the normal one. The intravenous injection of sodium chloride solution caused no change in the rate of secretion in the normal, but markedly increased the rate of the secretion in the transplanted organ.

The composition of urine secreted by the transplanted kidney differed somewhat from that secreted by the normal one. The constituents were similar, but the chlorides appeared to be more abundant in the urine from the transplanted kidney, while the organic sulphates, pigments and urea were more abundant in the urine from the normal organ.

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THE state legislature of Florida during its recent session, April 4 to June 2 of the present year, enacted a measure, commonly known as the 'Buckman Bill' designed by its originators to consolidate and strengthen, and to economize in the running expenses of the educational system of the state. By the provisions of the bill the entire system of higher education, consisting of a state university, a girls' college, and including the normal school for colored students and the institute for the deaf and blind, is under the management of a single board of control of five members appointed by the governor from five sections of the state. By the terms of the bill existing state schools are abolished as follows: The University of Florida, Lake City; Florida State College, Tallahassee; Normal School, DeFuniac Springs; East Florida Seminary, Gainsville; South Florida College, Bartow; Florida Agricultural Institute, Osceola County; and the Normal and Industrial department maintained by the state in the St. Petersburg Normal and Industrial School. To replace these abolished institutions there is created a University of the State of Florida

A Note on Transplantation of the Whole Liver in Dogs

Encouraged by the work on transplantation of the kidney in the dog and man, we have attempted to evolve a satisfactory technique for liver transplantation. For the past 15 months we have been homotransplanting whole livers with some success. Our most recently developed technique allows us to say that transplantation of the entire liver is possible in dogs and that the transplants function. To-date we have attempted 47 transplants with 14 successes. With recent innovations in technique, we have been successful in the last 4 out of 6 transplantations. Our criteria for a successful transplantation have been two: production of bile by the transplanted liver for at least 4 days, and survival of the recipient dog for a minimum of 5 days. We have other evidence that the liver homotransplants function.

As in any whole organ transplant, one must reinstitute a satisfactory arterial inflow and venous exit. The liver's great susceptibility to anoxia makes the rapid reinstitution of the blood supply a particularly important factor. Our first technique employed arterialization of the portal vein as the sole source of the blood supply for the transplant. However, this method of arterialization of the liver did not prove to be satisfactory and we changed to a method in which the liver was arterialized via the hepatic artery. Subsequently, we have found that the incidence of survival is higher if the portal vein and the hepatic artery are both reanastomosed to a venous and arterial supply respectively.

We have found that in a successful transplant, bile can be collected from the cannulized gall bladder of the transplant for about 4 days. Usually after the fifth day, only a small amount of bloody mucinous material issues from the cannula. In animals sacrificed at 6 days, gross degeneration of the transplant is usually evident. In some instances the liver seems partially viable up to 10 days, although it does not secrete bile. At 4 weeks, only a small shrunken remnant of barely recognizable liver tissue is found in those animals which survive.

As in kidney transplantation, the immunological host reaction seems to precede any gross vascular occlusion factor. On post mortem examination at 6 days, the transplanted liver shows obvious focal necrosis with all the anastomoses patent. Microscopically, the first indication of reaction is a periportal infiltration by round cells and polymorphonuclear cells. This reaction inexorably progresses to a marked generalized necrosis.

Believing that bacterial contamination of the canine liver played a prominent part in transplant survival, we attempted in early experiments to sterilize the liver of the donor dog with broad spectrum antibiotics given each day for at least 10 days prior to transplantation. However, we have come to doubt that it is possible to sterilize the canine liver consistently because of the great variety of organisms which seem to inhabit it. More importantly, we have found that liver transplants will survive without any attempt at sterilization, providing that the period of anoxia is minimal, and post operative antibiotics are given.

Whole liver transplantation may prove to be a useful technique in the further study of homologous whole organ transplantation. In addition, it may prove a valuable adjunct to the study of liver failure and liver function and it is in this area that we are working. Further work is being done on liver transplantation in other species in our laboratory. It is interesting to speculate about the possibility of transplanting the human liver in part or as a whole. The two problems of donation and technique of operation seem by themselves to be insurmountable obstacles with our present knowledge. Nevertheless, if some kind of assistance from a donor liver were available to patients in liver failure it would serve a most useful purpose. The liver has great "come back power" both in acute and chronic failure, making the idea of temporary supplementation of liver function by perfusion of a donor liver most attractive.

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